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**Investigation of the Replacement of Margaret
River Hairy Marron *Cherax tenuimanus* (Smith)
by Smooth Marron *C. cainii* Austin.**

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B.Sc. (Zoology), University of Western Australia

November 2004

A thesis submitted in partial fulfilment of the requirements for the degree of Master of
Science in Environmental Management.

Centre for Ecosystem Management
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ABSTRACT

The Margaret River hairy marron, *Cherax tenuimanus* (Smith, 1912) (Decapoda: Parastacidae) is critically endangered due to the introduction of the widespread marron, *C. cainii* Austin, 2002. This project investigates the rapid replacement of *C. tenuimanus* with studies important to its conservation.

The ability to identify correctly in the field *C. tenuimanus*, *C. cainii* and hybrids was investigated by linking morphology and marker allozyme loci. *C. tenuimanus* was readily identified in the field and errors were conservative; no genetically identified hybrids or *C. cainii* were field identified as *C. tenuimanus* during tissue samples collection. A prototype field identification guide has been constructed, aiming to provide the general public with an ability to identify correctly in the field *C. tenuimanus*. This should allow community participation in recovery plans centred on active removal of *C. cainii* and hybrids and ensure minimal accidental removal of *C. tenuimanus*. The guide will serve to allow recreational marron fishing within *C. tenuimanus* habitat, and raise public awareness about *C. tenuimanus* and its conservation.

For the purpose of investigating the role of hybridisation in the replacement, accurate field identification of hybrid marron, based on morphology, was not achievable. Investigation into the relationship between median carina length and orbital carapace length recorded during tissue sample collection provided some distinction, but not at a diagnostic level. The genetically diverse F₂ or backcross hybrid marron had the lowest accurate field identification. Further investigation into morphological and morphometric relationships will be necessary if precise accuracy in field identification of marron is deemed possible.

The distribution of marron species within Margaret River was mapped with *C. tenuimanus* found almost exclusively in the forested upper reaches, only in sympatry with *C. cainii*. An abrupt boundary of occurrence exists for *C. tenuimanus* between agricultural land use and state forest. A very small population of *C. tenuimanus* exists in the extreme lower reaches. It is proposed that extrinsic factors associated with the middle and lower reaches (changed water flows, presence of shelter, water pollution, fishing pressure, etc.) might account for the increased replacement of *C. tenuimanus*.

Repeated mark-recapture within a sympatric population aimed to investigate replacement mechanisms based on differences in intrinsic factors. Although not overly powerful, results suggested *C. cainii* has a greater growth rate and earlier spawning

period, both of which are attributes known to influence freshwater crayfish replacements elsewhere in the world.

A conceptual flowchart based on interacting intrinsic and extrinsic factors that influence the replacement of *C. tenuimanus* by *C. cainii* was constructed. This flowchart provides guidance in future research on replacement mechanisms and recovery actions for *C. tenuimanus*.

C. tenuimanus was nominated as critically endangered to the Western Australian Department of Conservation and Land Management. This was accepted and is currently awaiting final Ministerial approval prior to formal gazettal. An Interim Recovery Team has been established and is led by the Department of Fisheries.

Management recommendations are made with an emphasis on prioritising actions providing the most immediate and long-term advantage for *C. tenuimanus*. The Margaret River community is considered to be an important component in the overall recovery process.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

- (i) incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education;
- (ii) contain any material previously published or written by another person except where due reference is made in the text; or
- (iii) contain any defamatory material.

Signed

John J. S. Bunn

30/11/04

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Chapter 1: Introduction



The Margaret River Hairy Marron, *Cherax tenuimanus* (Decapoda: Parastacidae)

1.1 The Margaret River Hairy Marron

1.1.1 Background History

Marron are known to be the largest members of the genus *Cherax*, which belong to the Southern Hemisphere family of freshwater crayfish, Parastacidae (Austin and Knott 1996). Along with *C. quinquecarinatus* (Gray), *C. crassimanus* Reik, *C. preissii* Erichson and *C. glaber* Reik (as outlined in Austin and Knott (1996)) and another parastacid genus, *Engaewa*, marron are endemic to the south west of Western Australia. Due to their large size, marron have become a very desirable food source and an established aquaculture industry and recreational fishery have evolved around them. The distribution of marron now exceeds its former range in the south west of WA through translocation into farm dams and natural waterways (Morrissey 1978, Austin and Knott 1996). Morrissey (1978) provides anecdotal evidence of marron translocations throughout the southwest in the early to mid 1900s. These freshwater crayfish have also been translocated to other parts of Australia and the world for use as an aquaculture species (Horwitz 1990).

The original taxonomic description of marron, in 1912, named a single species, *C. tenuimanus*, with the Margaret River as the type locality (Smith 1912). Until recently, the taxonomy of marron has not been in dispute, however, this changed with the description of a new species: *Cherax cainii* (Austin and Ryan 2002). The species was reported to be the widespread and exploited form alluded to in the paragraph above while *C. tenuimanus* was found only within a single river system, the Margaret River. This formal taxonomic change is now widely accepted. Based on morphological features (see below) *C. tenuimanus* is commonly known as the Margaret River 'hairy' marron. When being discussed alongside *C. tenuimanus*, *C. cainii* is now generally referred to as the 'smooth' marron. Allozyme research over a 19-year period (Austin 1979, 1986, Austin and Knott 1996) documented the genetic uniqueness of *C. tenuimanus* and also the introduction of *C. cainii* into the Margaret River. Reproductive interactions within the Margaret River, determined from genetic (allozyme) data, indicated limited interbreeding and contributed to the justification of two separate species (Austin and Ryan 2002). As the original description of marron used specimens that were collected from the Margaret River, *C. tenuimanus* was retained for the unique species found only in this waterway (Austin and Ryan 2002). The widespread species was the one that received the new name of *C. cainii* based on the taxonomic rules set out in the International Code of Zoological Nomenclature (ICZN 2000).

The genetic difference between *C. tenuimanus* and *C. cainii* based on allozymes is in respect to three peptidase (*Lgg*, *Lp* and *Lt-2*) loci and one esterase (*Est*) locus (Austin and Ryan 2002). At all four of these allozyme loci, *C. tenuimanus* is fixed for a slow (S) allele while *C. cainii* is in part fixed for a fast (F) allele, as the S allele for *Est* was discovered at low frequency (0.10) in marron (*C. cainii*) sampled from the Blackwood River in 1985 after it was thought to be unique to the Margaret River (Austin and Ryan 2002). These allozyme differences now serve as genetic markers to identify the different species and also hybrids of the two. Genetically identified hybrids have been collected from a site where the introduction of *C. cainii* into the Margaret River and its subsequent complete replacement of *C. tenuimanus* in this location has been documented (Austin and Ryan 2002). Marron believed to be F₁ hybrids can be identified based on them being heterozygous (FS) at all four marker allozyme loci. Marron found to have a combination of homozygous (FF or SS) and heterozygous loci are either an F₂ hybrid or the offspring of a backcross.

Morphologically, *C. tenuimanus* is also distinct from *C. cainii* and can be distinguished predominantly on the basis of two particular traits. The median carina, the central ridge on the carapace, is continuous to the cervical groove in *C. tenuimanus*. In *C. cainii* the median carina does not extend to the cervical groove but is raised more prominently than in *C. tenuimanus*. The carapace, and to a lesser extent the abdomen (tail), are covered with clusters of setae in *C. tenuimanus*, hence the name Margaret River hairy marron. These clusters of setae are lacking in *C. cainii*, giving it the "smooth" appearance and its current common name. These morphological differences are depicted in Figure 1.1; however, a more comprehensive comparison between *C. tenuimanus* and *C. cainii*, including less prominent traits, can be found in Austin and Ryan (2002).

Prior to the description of two species, Nguyen *et al.* (2002), through mtDNA research, also found that, apart from the Margaret River story, there was a broad lack of genetic variation found within marron. Two separate haplotypes were sequenced from the Margaret River with one found only in this river and now known to represent *C. tenuimanus* and the other representative of the widespread *C. cainii*. Austin and Ryan (2002) and Nguyen *et al.* (2002), through their allozyme and mtDNA research respectively, demonstrate that *C. tenuimanus* represents about half of the genetic diversity found within marron.

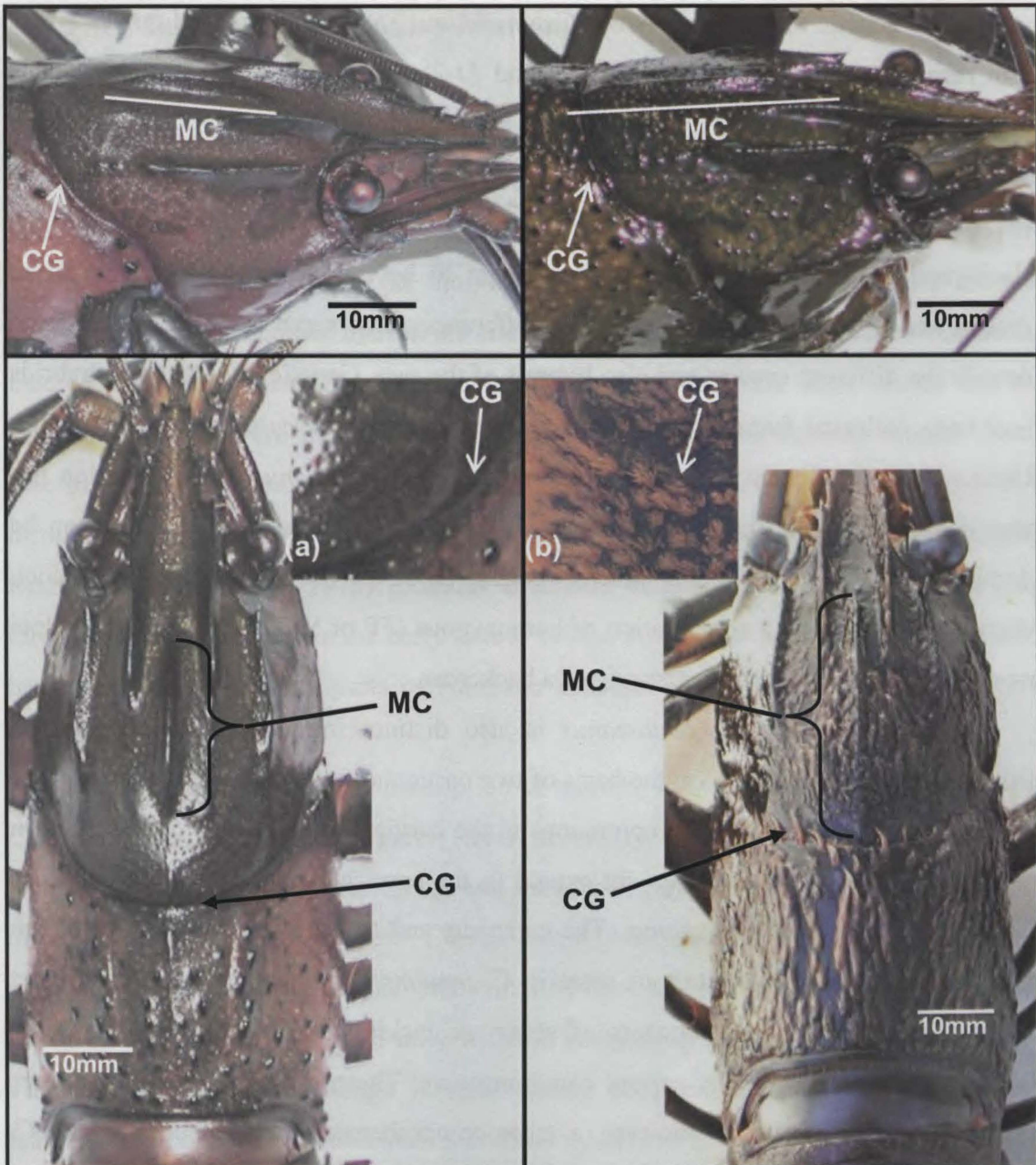


Figure 1.1. Basic morphological differences between *Cherax cainii* (left top and bottom) and *Cherax tenuimanus* (right top and bottom). The features to note are as follows: The median carina (MC) does not extend to the cervical groove (CG) in *C. cainii*, but is more strongly raised than in *C. tenuimanus*. The carapace of *C. tenuimanus* has clusters of setae. The 'smooth' carapace of *C. cainii* can be seen in close-up (a) while the 'hairy' carapace of *C. tenuimanus* is seen in close-up (b), which is an underwater shot.

Imgrund (1998), utilising microsatellite loci, also identified the Margaret River hairy marron as being genetically unique in a population genetic analysis of marron throughout southwestern WA. Two morphotypes were identified in the Margaret River, with the upper reaches having only one hairy form present, now known to be *C. tenuimanus*. The lower reaches had both morphotypes, with what is now known to be *C. cainii* also being present. Imgrund (1998), however, believed that the hairy morphotype of marron had been translocated to the lower reaches where it was interbreeding with the other morphotype and was diverging and becoming more genetically similar to the

lower reaches morphotype in this region. It was also concluded by Imgrund (1998), based on the microsatellite loci, that there was a significant level of genetic subdivision among populations of marron from other river systems.

1.1.2 Conservation Concern

Great concern is raised by Austin and Ryan (2002) and Nguyen *et al.* (2002) regarding the conservation and survival of the Margaret River hairy marron. Austin and Ryan (2002) recommended a thorough survey of the Margaret River to locate any remnant populations of *C. tenuimanus*, which they feared are threatened with extinction in the wild unless urgent conservation measures are undertaken. They suggested that, at the least, a captive population be established containing genetically uncontaminated *C. tenuimanus* in order to serve as a source for re-introduction of this species into the Margaret River. Regardless of any future taxonomic recognition, Nguyen *et al.* (2002) regarded the two genetically distinct forms of marron as representing 'evolutionary significant units', requiring a well structured conservation strategy to protect that status. The importance of conservation directed towards the hairy marron has been highlighted as being critical due to it being restricted to the Margaret River and the belief that this form represents the only significant genetic diversity within marron (Nguyen *et al.* 2002).

Since the presumed introduction of *C. cainii* into the Margaret River some time in the early 1980s, this species has spread rapidly (Austin and Ryan 2002). The rapid spread of *C. cainii* within the Margaret River system has been at the expense of *C. tenuimanus*, with this latter species being replaced in populations as the former has increased its abundance. Austin and Ryan (2002), through allozyme data, document the introduction of *C. cainii* at a site within the Margaret River just upstream of the townsite in 1985 and then the complete replacement of *C. tenuimanus* at this site by 1998.

The precise location and source of the introduction of *C. cainii* into the Margaret River remains speculative. Farm dams within the Margaret River catchment stocked with *C. cainii* could have been a source. If such a dam(s) had overflowed during a high rainfall period then it is possible that some *C. cainii* escaped, and if situated on a tributary there is a significant chance that these escapees ended up in the Margaret River. Deliberate translocation of *C. cainii* into the Margaret River is another possibility. This could have been done by someone attempting to restock a section of the river for the purpose of increasing a population size to enable a greater chance of catching a marron during the recreational fishing season. People moving between

different rivers during the recreational fishing season may have brought *C. cainii* to the Margaret River and then dumped them, possibly in fear of being caught with more than the legal daily bag limit for marron at the time. It may be that there have been several different translocation events of *C. cainii* into the Margaret River and also the possibility that these have continued to occur since the original introduction. Regardless of the actual method involved in the initial introduction of *C. cainii* into the Margaret River, it presumably involved a substantial number of marron to account for this species' rapid success. Whether it was multiple translocations or a single event that lead to a considerable quantity of *C. cainii* being introduced into the Margaret River is still not known.

In summary, *C. tenuimanus* represents the most important genetic and morphological difference in marron and has a highly localised distribution, being restricted to the Margaret River (Austin and Ryan 2002, Nguyen *et al.* 2002). In comparison, *C. cainii* remains relatively homogenous throughout its widespread distribution in southwestern WA (Austin and Ryan 2002, Nguyen *et al.* 2002), which is reflective of the large scale translocations that have occurred in this species (Morrissey 1978, Horwitz 1990). The Margaret River is no exception, and as a result *C. cainii* has been rapidly replacing *C. tenuimanus* and now threatens to cause the extinction of this unique species (Austin and Ryan 2002).

1.2 Replacement of Native Freshwater Crayfish Populations

1.2.1 Introduction of Non-Native Species

The replacement of a native freshwater crayfish by an introduced species is certainly not restricted to the Margaret River. Apart from the recent issues raised regarding *C. tenuimanus* and *C. cainii* within the Margaret River (Horwitz 1990, 1994, 1995, Imgrund *et al.* 1997, Imgrund 1998, Austin and Ryan 2002, Nguyen *et al.* 2002) and the introduction and potential spread of the yabby, *C. destructor*, into Western Australia (Austin 1985) this is a problem that has yet to become prevalent in Australia. However, it has definitely become an important subject of concern in other parts of the world.

In Europe there have been a number of deliberate introductions of non-native crayfish into natural waterways, typically the North American species *Orconectes limosus*, *Pacifastacus leniusculus* and *Procambarus clarkii*. This has largely been an attempt to replenish native crayfish populations that have been lost as a result of crayfish plague caused by the fungus, *Aphanomyces astaci* (Barbaresi and Gherardi 2000). It is believed that this pathogen may have entered Europe *via* Italy in the 1860s from infected crayfish that were released from the ballast of a North American ship (Barbaresi and Gherardi 2000). It has now spread across Europe severely reducing or wiping out native populations over a large area (Holdich 1988, Barbaresi and Gherardi 2000). In some countries it took a long time before the crayfish plague reached their crayfish populations (Holdich 1988). The North American species introduced to compensate for the loss of native populations have also furthered the decline of native species. These introduced species are known to be resistant to the crayfish plague fungus but can still carry and spread this pathogen to susceptible native European species (Holdich 1988, Lodge *et al.* 2000). Apart from spreading the crayfish plague fungus, North American species have also been demonstrated to have the ability to replace native populations through biological differences (Söderbäck 1995, Vorburger and Ribi 1999, Stucki 2002). *P. leniusculus* has also been shown to have the potential to impact negatively on aquatic macrophytes and macroinvertebrates (Nyström and Strand 1996, Nyström *et al.* 1999).

North America is home to more species of freshwater crayfish than anywhere else in the world. The threat to native populations of crayfish comes not from other continents but from the spread of species found within North America (Lodge *et al.* 2000). Several species translocated outside their natural range have been replacing native populations of crayfish (Lodge *et al.* 2000). Some species have also had

detrimental effects on the overall ecosystems they have been introduced into, such as impacting on other macroinvertebrates and reducing the amount of macrophytes present (Lodge and Lorman 1987, Olsen *et al.* 1991, Lodge *et al.* 1994, Charlebois and Lamberti 1996, Houghton *et al.* 1998). Much of the spread of crayfish species outside their natural range in North America has been blamed on their use as live bait for recreational fishing (Lodge *et al.* 2000).

Japan is home to only one native species of freshwater crayfish, *Cambaroides japonicus*; however, two North American species, *Pacifastacus leniusculus* and *Procambarus clarkii* have been introduced (Kawai *et al.* 2002). As a result of the introduction of least *P. leniusculus*, Japanese authorities have classified *C. japonicus* as endangered, due to the replacement of this native species (Kawai *et al.* 2002, Nakata and Goshima 2003).

1.2.2 Replacement Mechanisms

Many studies have attempted to evaluate the actual mechanisms behind the replacement of native crayfish by introduced crayfish. There are a number of attributed factors that have received notoriety in contributing to these replacements.

One of the concepts in crayfish replacements is that invaders are simply better competitors for resources, to the detriment of native species (Capelli and Munjal 1982, Butler and Stein 1985, Quinn and Janssen 1989, Hill *et al.* 1993, Hill and Lodge 1994, 1999, Söderbäck 1995, Vorburger and Ribi 1999, Usio *et al.* 2001, Nakata and Goshima 2003). The superior aggression of introduced species has received much attention in the replacement of native species. Laboratory studies have demonstrated how introduced species often dominate similar sized individuals of native species for resources such as shelter and food. The North American species, *Orconectes rusticus* is an introduced species in several states of the USA. Capelli and Munjal (1982) demonstrated, in laboratory experiments, how *O. rusticus* was aggressively dominant for shelter over *O. virilis*, a native, and *O. propinquus*, also introduced. Guiasu and Dunham (1999) found that *Cambarus robustus* had a strong dominance in agonistic contests over *C. bartonii bartonii*, which had sometimes been replaced following recent range expansion of *C. robustus* in Ontario, Canada. This was believed to be possibly due to the potential ability of *C. robustus* to competitively exclude *C. b. bartonii* should the two species be competing for a limited resource (Guiasu and Dunham 1999). Söderbäck (1991) conducted aggression experiments in Sweden involving the introduced North American *Pacifastacus leniusculus* and the native *Astacus astacus* and found *P. leniusculus* to be

dominant. If there were competition for a limited resource then *P. leniusculus* might be able to competitively exclude *A. astacus* (Söderbäck 1991). In Japan, the native *Cambaroides japonicus* has been shown via laboratory experiments to be subordinate to the introduced *P. leniusculus* in competition for shelters (Usio *et al.* 2001, Nakata and Goshima 2003).

The advantage of dominance in aggressive interactions of an introduced crayfish species over similar sized individuals of native species can be seen readily in competition for a limited resource. However, as larger sized crayfish can be expected to win agonistic encounters (Pavey and Fielder 1996), a species that obtains a greater overall size would be likely to have an overall advantage in resource competition. In laboratory experiments on aggressive dominance between non-native *P. leniusculus* and native *Austropotamobius torrentium*, there was no species that was dominant when crayfish size was equal (Vorburger and Ribi 1999). Due to the faster growth rate and larger size obtained by *P. leniusculus*, it was expected it would have the advantage in natural environments (Vorburger and Ribi 1999). Butler and Stein (1985) came to the same conclusion that the larger non-native *O. rusticus* would dominate over the smaller native *O. sanborni* in this species' natural habitat, after experiments between similar sized crayfish revealed neither species to be dominant.

This size advantage does not have to apply solely to the size obtained by adults but can also be related to a faster growth rate in young-of-year (YOY) crayfish. Superior growth rate in young-of-year (YOY) for an introduced species can lead to greater recruitment of juveniles into populations caused by such factors as continued size advantages in the competition for resources (Butler and Stein 1985). Even if growth rates of YOY between an introduced and native species are not significantly different the actual timing of their production will be important. YOY of one species produced shortly after those of another would be likely to have continuous size disadvantage in the competition for the same resources (Rorer and Capelli 1978).

Differential susceptibility to predation is another process that has received wide attention in the replacement of native crayfish species. Introduced species may be less prone to predation due to larger size (adults and YOY), which can influence competitive ability for shelters and size related predation risk and also behavioural preference for shelters, which can affect the risk of predation (Butler and Stein 1985, Quinn and Janssen 1989, Söderbäck 1992, 1994a, 1995, Didonato and Lodge 1993, Garvey *et al.* 1994, Hill and Lodge 1994, 1999). Increased predation of native species over

introduced species could readily contribute to their replacement by reducing both the adult population size and recruitment potential of YOY.

Life history traits of introduced crayfish species are another consideration. Several introduced crayfish species have been shown to have the ability for greater population growth due to factors such as an earlier onset and larger size at maturity, earlier release of young and greater egg production (Butler and Stein 1985, Söderbäck 1995, Distefano *et al.* 2002, Stucki 2002).

Inappropriate mate selection and hybridisation between native and introduced crayfish in sympatric populations have also received attention. Hybridisation has been instigated as increasing the rate of replacement of *O. propinquus* by *O. rusticus*, above that of other documented mechanisms (Perry *et al.* 2001a, 2001b, 2002). Butler and Stein (1985) outline how the preference for female *O. rusticus* by male *O. sanborni*, when the two species are in sympatry, could reduce the reproductive success of both species. Female *O. rusticus* may suffer from reduced fecundity or production of non-viable hybrids as a result of interspecific matings and *O. sanborni* would be equally affected by a reduction in intraspecific matings. It was suspected these interactions contributed to a 90% reduction in recruitment for both species in sympatry compared to allopatry (Butler and Stein 1985). However, *O. rusticus* was expected to progressively increase in abundance in sympatry due to the greater conspecific mate selection and the superior offspring production capabilities of females compared to *O. sanborni* (Butler and Stein 1985).

Reproductive interference between native *A. astacus* and introduced *P. leniusculus* was suggested to influence the replacement of the native species (Söderbäck 1994b, 1995). A reduced recruitment potential due to interspecific mating of female *A. astacus*, which cannot hybridise with *P. leniusculus*, was expected not to be a problem when both species were equally abundant (Söderbäck 1994b). However, as the proportion of *P. leniusculus* increases so could the rate of replacement due to a higher frequency of interspecific matings involving female *A. astacus*, which would have a severe effect on the recruitment ability of this native species (Söderbäck 1994b).

1.2.3 Interacting Factors

There are many interacting factors that have encompassed the perceived basis of crayfish replacement. These factors can be categorised essentially as being primarily intrinsic, which applies to properties that are inherent to the particular species, or extrinsic, pertaining to the external environment and properties not directly associated

with the particular species. This concept is adapted from Bergmann and Moore (2003) who describe how intrinsic and extrinsic factors interact to affect intraspecific aggression in field observations of *O. rusticus* and *O. virilis*. Intrinsic factors, such as the size of individuals, interacted with extrinsic factors, such as the particular resource, to determine the degree of aggression. The most valuable resource was shelters, which produced the longer and more intense level of competition, given the contesting pair were of similar size. This was followed by detritus, which was believed to be apparently less valuable than shelters but more valuable than macrophytes, another food source that had the lowest level of competition intensity. Although Bergman and Moore (2003) were describing factors affecting intraspecific aggression, this can be directly related to crayfish replacement in terms of differing competitive ability between introduced and native species.

An important consideration in referring to a greater competitive ability for resources of introduced species, an intrinsic factor, is whether or not these resources are actually limited, which is an extrinsic factor. If a particular habitat has an abundance of food and shelter, competition is likely to be minimal (Butler and Stein 1985, Bergman and Moore 2003), which then requires another explanation for the replacement of a native crayfish species. The concept of interacting intrinsic and extrinsic factors can also be applied to other mechanisms that have been used to explain the replacement of native crayfish by introduced species, such as the influence of predators, life history trait differences and reproductive interactions.

The extrinsic factors may often control the degree to which a particular intrinsic factor contributes to the replacement of a native species. A species with the greater capacity for population growth and recovery after population reductions (intrinsic) will have the upper hand in situations where particular extrinsic factors bring about an advantage in having this ability. The same theory applies to having a greater competitive ability (intrinsic) where one species can competitively exclude another from limited resources (extrinsic) to the extent that the survival of this species is compromised due to other possible interacting intrinsic and extrinsic factors.

When studying native crayfish replacements it will be essential to investigate intrinsic and extrinsic factors and their potential interactions, which can be likened to positive and negative feedback loops. Certain aspects can potentially work positively for the establishment of an introduced species and negatively against the persistence of a native species. Experiments focussing on hypotheses based on intrinsic and extrinsic factors believed to be, or possibly be, influencing the replacement of a native crayfish

will be needed to draw conclusions as to why this process is occurring. A conceptual flowchart based on interacting intrinsic and extrinsic factors has been developed in Figure 1.2 to demonstrate how these factors may affect the recruitment and population growth potential of freshwater crayfish species. From this flowchart it will be possible to generate the hypotheses needed in studies directed at comparing differences in intrinsic factors between freshwater crayfish species, and how they are influenced by extrinsic factors, to determine the mechanisms responsible for the replacement of one species by another.

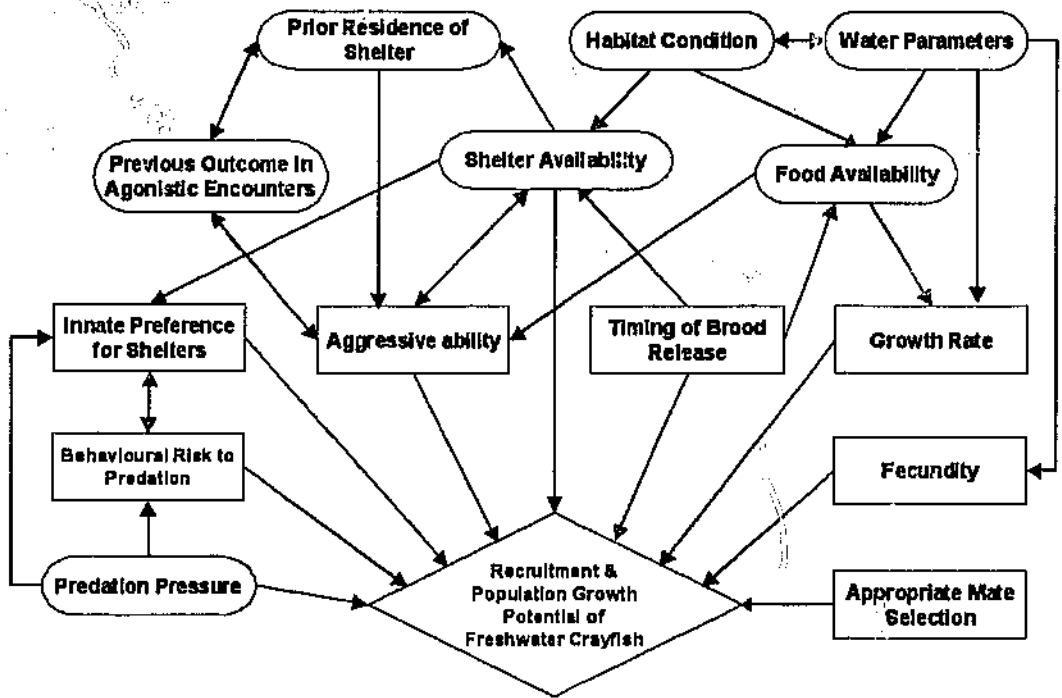


Figure 1.2. Conceptual flowchart of the intrinsic and extrinsic factors that influence the recruitment and population growth of freshwater crayfish. The rectangles represent the intrinsic factors, which are the properties inherent to particular crayfish species. The oblong circles represent extrinsic factors, which are the properties pertaining to the external environment and properties not directly associated with particular species. The arrows represent the direction of influence between factors, with some having a bilateral effect on each other. Extrinsic factors can have an affect on other extrinsic factors, but are more likely to have an affect on the intrinsic factors, in terms of their level of influence in the recruitment and population growth of species.

1.3 Project Outline

1.3.1 Importance

Preventing the extinction of *C. tenuimanus* will ensure that almost 50% of the known genetic diversity in marron is preserved (Austin and Ryan 2002, Nguyen *et al.* 2002). At present, *C. tenuimanus* is the only member of the genus *Cherax* found in southwestern Western Australia that is considered to be a short-range endemic species (Harvey 2002). Its protection will also help to uphold the overall high level of biodiversity found in southwestern Western Australian and ensure that this species is not added to the comparable list of extinct fauna that exists for the entire state (Hobbs and Mooney 1998).

The overall aim of this project is to formulate directions for the conservation of *C. tenuimanus* and evaluate possible replacement mechanisms operating for the two marron species within Margaret River. Essential to this is determining the present distribution of *C. cainii* and *C. tenuimanus* within the Margaret River and exploring differences in intrinsic factors between the two species and how these may be affected by extrinsic factors. As so little is known about *C. tenuimanus*, the information gathered should prove valuable in determining the conservation status of this species.

1.3.2 Chapter Content

Chapter 2: Field Identification of *C. tenuimanus*, *C. cainii* and Hybrids

Austin and Ryan (2002) have shown that hybrids may not persist in locations where *C. cainii* has completely replaced *C. tenuimanus* and that they also do not make up a significant proportion within sympatric populations. Although this may imply that hybridisation is not a major replacement mechanism, it still is important to evaluate the role the hybridisation process is having. Central to this will be the ability to correctly identify hybrids when monitoring marron populations within the Margaret River. The capacity to be able to do this in the field will be a valuable conservation advantage, as this will reduce the time and costs associated with genetic analysis utilising the allozyme marker loci.

The value of being able to correctly identify hybrids in the field is clear; however, the importance of ensuring that *C. tenuimanus* and *C. cainii* can also be identified should not be overlooked. Ensuring that *C. tenuimanus* can be accurately identified will provide an avenue for raising community awareness of the plight of this unique threatened species. The ability for the local community to participate in conservation actions aimed at removing *C. cainii* from the Margaret River will only be

guaranteed if *C. tenuimanus* can be correctly identified and therefore not accidentally taken by those people involved. This is fundamental in allowing the continuation of recreational fishing for marron in the Margaret River.

The identification in the field of *C. tenuimanus*, *C. cainii* and hybrid marron, based on their morphological differences, would be very useful. The four allozyme marker loci detailed in Austin and Ryan (2002) were used to test the accuracy of field identifications. Linking diagnostic genotypes to morphological and morphometric features was used to test the potential of producing a field identification guide to aid in the conservation of *C. tenuimanus*.

Chapter 3: Distribution of Marron Within the Margaret River and Mark-Recapture Study to Evaluate Replacement Mechanisms

An important component of being able to guide conservation of *C. tenuimanus* will be to determine where remaining populations of this species are located, which was emphasised by Austin and Ryan (2002). Appropriately, such a survey of the Margaret River was undertaken in this study and will serve to guide important aspects in the conservation of *C. tenuimanus*. This distribution map of *C. tenuimanus* within the Margaret River details the extent of this species' population range and will be valuable in the assessment of its conservation status.

Another important component of the survey will be to determine the relative proportions of both species, and hybrids, found within populations, and how this might differ along the length of the Margaret River. The location of marron populations containing *C. tenuimanus* will serve to highlight areas of critical importance for directing management actions aimed at preventing the extinction of this species. Comparing areas where *C. tenuimanus* is present and absent will provide clues to some of the extrinsic factors contributing to the replacement of this species by *C. cainii*. This is in respect to factors such as habitat condition and/or the associated land use of particular areas that appear to influence the distribution of *C. tenuimanus* and its proportion within populations.

Mark-recapture studies provide the capacity for the investigation of intrinsic factors related to each marron species. Through conducting a repeated mark-recapture study of a sympatric population of *C. tenuimanus* and *C. cainii*, comparisons between intrinsic factors were explored. This study also aimed to test particular hypotheses

related to differences in intrinsic factors that could be contributing to the replacement of *C. tenuimanus* by *C. cainii*.

By recapturing marron, an estimate of growth rate can be produced based on the change in size from the initial capture and the subsequent time interval. *C. cainii* is hypothesised as having a greater growth rate, which would benefit this species through such factors as an increased offspring production potential and a size advantage in agonistic interactions. The movement of marron within populations can also be tracked in the same manner. *C. tenuimanus* is hypothesised as showing a greater degree of movement, related to an increased need to search for resources such as food and shelter due to a better competitive ability of *C. cainii*.

By conducting such a study over several sampling events then changes in the overall population can be noted. These include some life history traits such as the time of year spawning occurs or when broods are released from their mothers. *C. cainii* is hypothesised as releasing its brood earlier, giving this species a continued size advantage in the competition for resources that juveniles of both species may be competing for. This scenario could then transpose into an increased recruitment potential of *C. cainii* due to a greater juvenile survival compared to *C. tenuimanus*.

Chapter 4: Synthesis and Recommendations for Management

Once possible intrinsic and extrinsic factors were identified (Chapter 3) it was possible to adapt the conceptual flowchart of Figure 1.2 to what is occurring in the Margaret River. This exercise served to highlight the further experimentation that may be required into factors not identified in these studies, which may be influencing the replacement of *C. tenuimanus*. Factors that have been suggested by these studies may also need experimental confirmation to give support to their role as replacement mechanisms. Direct results from the studies undertaken and the identified future research should provide a basis on which to direct both the short and long-term management strategies required in the conservation of *C. tenuimanus*. Those involved, and their role, in the conservation of *C. tenuimanus* can be identified.

Chapter 2: Field identification of marron within the Margaret River



An F₁ hybrid collected from the forests of the upper reaches of the Margaret River where the endemic hairy marron exists in sympatry with the introduced smooth marron.

2.1 Introduction

2.1.1 History of Marron in the Margaret River

C. cainii and hybrid marron were first detected in the Margaret River in 1985 from a site (approximately 4 km ENE of centre of townsite) that previously contained only *C. tenuimanus* (Austin and Ryan 2002). From a sample of 54 marron, 45 had *C. tenuimanus* morphologies and were homozygous for the slow allele at all four marker allozyme loci (*Lt-2*, *Lgg*, *Lp* and *Est*). Five marron had morphological features of the widespread *C. cainii*, which had not previously been found in the Margaret River. At all three peptidase marker loci these marron were homozygous for the fast allele, but for the *Est* marker locus they were polymorphic. As the slow allele had been found at low frequency at the *Est* locus in marron from the Blackwood River, these five marron were all considered to be *C. cainii* (Austin and Ryan 2002). The four remaining marron had morphologies intermediate to that of *C. tenuimanus* and *C. cainii*. Of these, three were considered to be likely F₁ hybrids as they were heterozygous at all four marker loci. One marron was heterozygous at all three peptidase marker loci and homozygous for the fast allele at the *Est* locus, so was considered a likely F₂ or backcross hybrid (Austin and Ryan 2002).

This same site was sampled again in 1992 and 1998 where a drastic change in the type of marron present was documented (Austin and Ryan 2002). In 1992, 40 marron were sampled with only three being genetically identified as *C. tenuimanus* and 32 genetically identified as *C. cainii*. Five marron were genetically identified as F₂ or backcross hybrids with no F₁ hybrids collected (Austin and Ryan 2002). In 1998, 66 marron were sampled and all were considered to be *C. cainii*. All were homozygous for the fast allele at the three peptidase marker loci. The frequency of the fast allele at *Est* was not significantly different from marron sampled within the Blackwood in 1985 (Austin and Ryan 2002). No *C. tenuimanus* or hybrid marron were genetically identified at this site in 1998.

Many more hybrid type marron were expected to have been collected from this site over the repeated sampling events under the premise that random mating was taking place (Austin and Ryan 2002). Austin and Ryan (2002) attributed this to positive assortative mating by *C. tenuimanus* and *C. cainii* or reduced hybrid viability. It was this lack of genetic introgression caused by minimal interbreeding between *C. tenuimanus* and *C. cainii* that gave Austin and Ryan (2002) their justification for recognising the Margaret River hairy marron and the widespread smooth marron as separate species.

2.1.2 Hybridisation Problems

Austin and Ryan (2002) believe that interbreeding between *C. tenuimanus* and *C. cainii* is minimal. Although this implies that hybridisation is uncommon, it is still important to determine the degree to which this process could be contributing to the replacement of *C. tenuimanus* by *C. cainii*. Until the viability of hybrids and the reproductive behavioural interactions between *C. tenuimanus* and *C. cainii* are known, hybridisation, as a replacement mechanism, cannot be overlooked.

The low proportion of hybrids present in populations does not mean that there are not significant reproductive interactions occurring between *C. tenuimanus* and *C. cainii*. Interspecific matings can represent reproductive interference by reducing the recruitment success of both species (Capelli and Capelli 1980, Smith 1981, Berrill 1985, Butler and Stein 1985, Söderbäck 1994b). Copulation with interspecific partners, related to inappropriate mate choice by males and females for each species, may decrease the fecundity of females (Berrill 1985, Butler and Stein 1985, Söderbäck 1994b). The proportion and types of hybrids present may have the potential to serve as an indicator of the amount of reproductive interference occurring between *C. tenuimanus* and *C. cainii* in sympatric populations. This will be valuable information if reproductive interference proves to be a significant mechanism in the replacement of *C. tenuimanus* by *C. cainii*.

In North America, the role that hybridisation has played in the replacement of native *Orconectes propinquus* populations by introduced *O. rusticus* has been investigated through the genetic identification of hybrids (Perry *et al.* 2001a, 2001b, 2002). Hybridisation was estimated to increase the spread of *O. rusticus* genes up to 36% above documented ecological replacement mechanisms (Perry *et al.* 2001a, 2002). Contrasting hybridisation processes have been shown to occur between *O. rusticus* and *O. propinquus* in different habitats (Perry *et al.* 2002). This suggests that the role hybridisation plays, as a replacement mechanism, may be dependant on the particular environment (Perry *et al.* 2002). The viability of native crayfish populations, such as *C. tenuimanus*, can be potentially estimated through examination of the level of hybridisation occurring and the competitive ability of this native species within a particular habitat compared to introduced species (*e.g.* *C. cainii*) and hybrids (Huxel 1999, Perry *et al.* 2001a). Investigating the degree that hybridisation operates, as a replacement mechanism, allows for other potential mechanisms to be better evaluated.

2.1.3 Identifying Marron

Determination of the major replacement mechanisms is highly likely to form part of a recovery plan for the conservation of *C. tenuimanus*. Correct identification of hybrid marron in populations will therefore be a necessary procedure. Through the use of the allozyme markers identified in Austin and Ryan (2002), pure *C. tenuimanus*, pure *C. cainii* and their hybrids can be identified genetically. For this method the collection of tissue samples is needed, which requires the purchase and transportation of dry ice or liquid nitrogen to freeze samples if they are to be obtained in the field. Once collected and frozen, samples need to be transported back to the laboratory. Analysis of tissue samples can be very time consuming, and expensive laboratory equipment and chemicals, often quite dangerous, are needed. As time and money are often limited resources in threatened species recovery plans, accurate identification of hybrid marron in the field will be of great benefit. Field identification of marron will save time and money by reducing the need to take tissue samples. The ability to reduce expenses and save precious time will be a valuable asset in the development of recovery plans for *C. tenuimanus*.

One of the main purposes of this study was to investigate the feasibility of producing a guide that can be used by researchers and managers to accurately identify hybrids, pure *C. tenuimanus* and pure *C. cainii* in the field. This field identification guide will assist in an examination of the level of hybridisation within populations, in a time and cost effective manner. Field identification will need to centre on distinguishing hybrids from *C. tenuimanus* and *C. cainii* on the basis of diagnostic characters that can be easily interpreted or measured. As hybrids are not expected to make up a significant proportion of the marron present in populations (Austin and Ryan 2002), the level of accuracy required in such an identification tool will need to be precise. A small number of misidentifications could lead to a relatively significant misdiagnosis of the level of hybridisation occurring in populations. Because of this factor, a target level of accuracy should be set close to (and preferably at) 100%. Although this may seem overly rigorous, it is justified because of the importance of determining what role hybridisation plays in the replacement of *C. tenuimanus*.

The feasibility of producing such an identification guide was evaluated through an investigation into the level of accuracy achievable in the field identification of *C. tenuimanus*, *C. cainii* and hybrid marron. This involved the collection of tissue samples in the field from captured marron for electrophoretic analysis of the four allozyme marker loci to determine diagnostic genotypes. Prior to tissue sample collection, type

predictions (*C. tenuimanus*, *C. cainii* or hybrid) based on the diagnostic morphological differences were made. Accuracy was then determined by seeing if predictions matched the diagnostic genotypes for the marker allozyme loci. Morphometric features recorded were used to establish if they can serve as a way of accurately distinguishing the genetically identified marron types, and to improve the level of accuracy achievable in field type predictions. From these results it was possible to determine what identification tools need to be incorporated into a field identification guide to deliver the required level of accuracy.

Raising public awareness of the plight of *C. tenuimanus* should be an important facet of this species' conservation. One very effective technique to achieve this goal could be the promotion of active community participation in recovery plans. A successful recovery plan for the conservation of *C. tenuimanus* is likely to involve removal of the major threatening processes, which in this case will include removal of both *C. cainii* and hybrid marron. Community participation in the conservation of *C. tenuimanus* would be likely to involve contributing to the active removal of *C. cainii* and hybrids. *C. cainii* is a clear threat due to yet to be determined replacement mechanisms. Hybrids are also a threat by being a source of genetic contamination through backcrossing with *C. tenuimanus*, challenging its status as an 'evolutionary significant unit' (Moritz 1994, Nguyen *et al.* 2002).

As marron, in general, already have a high profile in southwest Western Australia due to recreational fishing and aquaculture, encouraging community participation should not be a difficult task. However, before community involvement can be considered, those involved will need to be given the practical knowledge of how to distinguish the basic morphological differences of marron present in the Margaret River. Therefore, another intention of this study was to determine the feasibility of producing an identification guide aimed at members of the general public to enable their involvement in community recovery plans and, as a consequence, raise wider public awareness of the plight of *C. tenuimanus*. The ability to produce this guide will be based on the analysis of diagnostic morphologies and genotypes as described above to ensure that *C. tenuimanus* can be correctly identified. The importance of this field identification not only applies to preventing the removal *C. tenuimanus* during community participation in recovery plans, but also during the recreational marron fishing season. The issue with recreational marron fishing is of particular importance in allowing this activity to continue within the remaining range of *C. tenuimanus*.

2.1.4 Field Identification

The overall aim of this study was to test the hypotheses that *C. tenuimanus*, *C. cainii* and hybrids can be correctly identified in the field based on their distinctive morphological features and secondly that morphometric characters can further distinguish between the different marron species and types of hybrids. This was tested by determining the diagnostic genotypes from the four marker allozyme loci (*Lt-2*, *Lgg*, *Lp* and *Est*) from electrophoretic analysis of tissue samples taken from captured marron. These genotypes were then referred back to type predictions (*C. tenuimanus*, *C. cainii* or hybrids) and morphological and morphometric data recorded when marron were captured in the field and tissue samples taken. From the results the feasibility was determined for the production of practical field identification guides for:

1. Scientists and environmental managers to:
 - a. Use morphological and morphometric characters to assist in correctly identifying in the field all forms of marron present in populations.
 - b. Allow an accurate and rapid evaluation of the hybridisation process within populations.
2. Members of the general public to:
 - a. Become aware of the threatened status of *C. tenuimanus*.
 - b. Participate in recovery plans by ensuring that *C. tenuimanus* can be correctly identified, therefore aiding the specific taking of only *C. cainii* and hybrids during active removal.
 - c. Correctly identify *C. tenuimanus* during the recreational marron fishing season.

2.2 Methods

2.2.1 Field identifications

Prior to the recording of morphometric features (see Chapter 3, Section 3.2.3), marron were identified as either *C. tenuimanus*, *C. cainii*, or hybrids based on visual interpretation of morphological characters that distinguish *C. tenuimanus* from *C. cainii* (see Chapter 1). Marron identified as *C. tenuimanus* had a median carina that was uniformly raised along its entire length and appeared continuous to the cervical groove, plus a carapace with clusters of setae. Small sized marron believed to be *C. tenuimanus*, due to a continuous median carina, generally only had the distinctive clusters of setae along the margin of the cervical groove. Marron with a median carina that had a prominently raised section not continuous to the cervical groove and smooth carapace lacking in seta were identified as *C. cainii*. These diagnostic characteristics were consistent across all sizes of marron identified as *C. cainii* in the field. Marron that appeared to possess intermediate morphologies between *C. tenuimanus* and *C. cainii* in relation to the degree of setation and/or appearance of the median carina were considered to be hybrids. Marron identified as hybrids were those that possessed clusters of small 'stubble like' setae, compared to the longer more distinctive setae of marron identified as *C. tenuimanus* (see Figure 1.1), AND/OR generally possessed a median carina that while not being continuous to the cervical groove was clearly longer in length than marron identified as *C. cainii* and may not have been as prominently raised. There may also have been for some individuals identified as hybrids a weakly raised section of the median carina that appeared continuous to the cervical groove following the terminal point of inflection of the strongly raised section of the median carina (see Box 2.1 for an example of this). Marron identified as hybrids may have had varying degrees of intermediate morphologies in relation to either the level of setation and the appearance of the median carina (see Box 2.2 for an example). No distinction was made between F_1 , F_2 or backcross hybrids when a marron was predicted to be a hybrid based on its morphology. It was considered that clear separation between these types could not be made until the link between diagnostic genotypes and morphological and morphometric features had been investigated.

2.2.2 Collection of marron tissue samples

In view of the stated aims, marron believed to be hybrids were targeted for tissue sample collection. Most tissue samples were obtained in the upper reaches of the Margaret River at sites where *C. tenuimanus* and *C. cainii* occurred in sympatry (see

Chapter 3). As a consequence of being targeted, hybrid marron were over-represented in the number of tissue samples collected, in relation to their actual proportion within populations (see Chapter 3). Samples were also obtained from a selection of marron identified as *C. tenuimanus* or *C. cainii* on the basis of morphology. At sites that appeared to consist entirely of *C. cainii*, tissue samples were obtained from a collection of marron to validate this assumption and to add to the data set linking morphology to genotype. Due to numerous sites that contained only *C. cainii* (see Chapter 3), there was another representative bias in the number of samples collected from this species compared to *C. tenuimanus* and hybrids.

Marron were sampled by causing individuals to autotomise a single walking leg (pereopod) by squeezing and then tugging on the leg with forceps. The leg was wrapped in aluminium foil with a label, the foil itself labelled and the prepared sample frozen in the field in liquid nitrogen. The second left walking leg was favoured, to remain consistent, but if this leg was missing from marron then another was chosen. If the marron sampled was particularly small, then a single claw was taken. Upon return to the laboratory, samples were stored at -85°C until electrophoretic analysis.

2.2.3 Electrophoresis

All marron were scored for variation at the four marker allozyme loci identified in Austin and Ryan (2002). These consist of three peptidase loci: L-Leucyl-glycyl-glycine (*Lgg*), L-Leucyl-proline (*Lp*) and L-Leucyl-tyrosine 2 (*Lt-2*), and Esterase (*Est*). *C. tenuimanus* is fixed for a slow allele (S) at each of these loci. *C. cainii* is generally considered as fixed for a fast allele (F) at all four loci except that the slow *Est* allele was found to be present at a low frequency in marron from the Blackwood River (Austin and Ryan 2002) (see Chapter 1, Section 1.1.1). After each marron's genotype was determined it was placed in one of four distinct groups. Those that were homozygous at all four loci for the slow allele (SS) were considered to be a purebred *C. tenuimanus*. Any marron homozygous at all four loci for the fast allele (FF) was considered to be a purebred *C. cainii*. Marron that were heterozygous at all four loci (FS) were considered F_1 hybrids. Those with a mixture of homozygous and heterozygous loci were considered as F_2 or backcross hybrids.

Two electrophoretic techniques were used to determine the genotypes of marron from the tissue samples that were collected in the field. Starch gel electrophoresis (SGE) was used for samples collected in March and August 2002 and carried out at the School of Ecology and Environment, Deakin University, Warrnambool Campus, Victoria.

Cellulose acetate gel electrophoresis (CAGE) was used for samples collected in October and December 2002 and carried out at the School of Natural Sciences, Edith Cowan University, Joondalup Campus, Western Australia. The CAGE technique was first carried out at Deakin University to rerun a selection of samples already scored using the SGE technique.

SGE Procedure

For each sample, an extract of muscle tissue was taken and homogenised in a solution consisting of 10 % sucrose, 0.1 % mercaptoethanol and 0.1 % bromophenol blue. An approximate ratio of 1:1 for volume of tissue to homogenising solution was used. Samples were either homogenised on a ceramic grinding tray or with fine forceps in a 1.5 ml eppendorf tube, which were then centrifuged for ten minutes at 13000rpm and 3 °C, producing a homogenate that could then also be utilised for CAGE and also stored at -85 °C for later use. Individual homogenate samples were transferred onto small pieces of blotting paper, which were then applied to the gel. Electrophoresis was carried out in horizontal gels of 12% Starch in 1xTEB (45 mM Tris, 1 mM EDTA and 25 mM borate, pH 8.0) buffer. The electrode buffer (continuous) was 4xTEB and gels were run at 350 V and 45 mA for roughly five and a half hours at 4 °C. After completion, gels were removed from the running tanks, sliced into three replicate portions and stained for the marker allozyme loci. Only three portions were needed because there was sufficient mobility difference between *Lp* and *Lgg* to allow both to be scored on the one gel. The *Est* stain consisted of 2.5 mM α -naphthyl acetate in 0.09 M tris-maleate buffer, pH 5.3 with 0.025 % (w/v) fast black salt added just prior to use. The *Lp* and *Lgg* stain consisted of 0.9 mM leu-pro, 1 mM leu-gly-gly, 0.8 mM o-dianisidine diHCL and 0.025 % (w/v) peroxidase in 0.1 M sodium phosphate buffer, pH 7.0 with 0.025 % (w/v) L-amino acid oxidase added immediately before use. The *Lt-2* stain consisted of 0.8 mM leu-tyr, 0.8mM o-dianisidine diHCL (dry ingredients of *Lt-2* and o-dianisidine were first dissolved in 2 drops of 0.01 M HCl) and 0.025 % (w/v) peroxidase in 0.1 M sodium phosphate buffer, pH 7.0 with 0.025 % (w/v) L-amino acid oxidase added immediately before use. All stains were mixed 1:1 with 2 % agar solution (maintained at 60 °C), and the specific stain mixtures spread over the gel portions. Each gel was placed in a drying oven at 36 °C until the allozyme bands become clearly visible. The gels were then scored for variation at the marker allozyme loci for which they were stained. Genotypes were determined for marron allowing them to be placed into the genetically identified type groups.

The amounts entered for ingredients in the stain mixtures were mostly approximations based on recipes derived from citations within Austin and Ryan (2002) and also those from Herbert and Beaton (1993) designed for CAGE analysis. Once an amount had been initially measured it was then visually approximated on the end of spatula when making up the stain mixtures.

CAGE Procedure

This electrophoretic technique was first carried out on a selection of samples that were already genotyped using SGE at Deakin University for the purpose of developing this method for use at Edith Cowan University. Samples used were those that had been homogenised and centrifuged as described above for the SGE technique. A four gel running tank was used with 11 samples per gel allowing each marron to be scored for all four marker loci per run. The gels used were 76x76 mm Titan® III Cellulose Acetate plates (Catalogue # 3033, Helena Laboratories, Beaumont, Texas U.S.A.). Gels were soaked prior to use in TG (250 mM tris and 1.9 M glycine) buffer, pH 8.5, which was also the tank buffer (i.e. a continuous buffer system). Samples were applied to the gel using a Helena Super Z-12 applicator kit (Catalogue # 4093). The sample well plates from the applicator kit were loaded with 10 µl of homogenate from each sample. Gels were run at 200 V and 2 mA/gel for roughly 25 minutes at room temperature. After completion of the run time, gels were removed, blotted dry and each one stained for a separate allozyme marker locus. The stain mixtures were the same ingredients and concentrations as that used for the SGE technique, with the exception that separate mixtures were prepared for *Lp* and *Lgg*. The volume of mixture required for the cellulose acetate gels was only a tenth of that needed for the starch gels. After the stain mixture was applied to the gels, they were covered in plastic wrap and placed in a drying oven at 36 °C until allozyme bands became clearly visible. Gels were then scored for variation at the four marker loci. The genotypes were compared to those derived for the same marron using SGE to ensure compatibility between the two techniques.

The same type of cellulose acetate gels, sample application, running buffer and stain mixtures used at Deakin University were used at Edith Cowan University (see above). The differences were as follows:

A 1:1 ratio of 0.09 M tris HCl, pH 8.0 grinding solution to muscle tissue volume and 10 µl of 0.1 % bromophenol blue was used to homogenate each sample in a 1.5 ml eppendorf tube. Samples were then centrifuged for five minutes at 13000 rpm at room

temperature and the homogenate pipetted into smaller eppendorf tubes that were labelled and stored at -85°C until electrophoretic analysis. A Titan Gel® chamber (Catalogue # 4063) was used to run 2 gels at a time at 200 V and 2 mA/gel at room temperature. Gels to be stained for *Est* and *Lp* were run together for 30 minutes and *Lt-2* and *Lgg* were run together for 25 minutes. 10 drops of saturated Fast Red TR salt solution was used in the stain recipe for *Est* instead of fast black, and like the fast black, was added just prior to use. Once scored for variation at the four marker loci, each marron was placed into one of the four genetically identified type groups.

The recipes used for the stain mixtures and other important media for the SGE and CAGE techniques are listed in Appendix 1.

2.2.4 Accuracy of field identifications

After genotyping the four marker allozyme loci, the accuracy of the field identification (*C. tenuimanus*, *C. cainii* and hybrids) based on morphology for sampled marron was investigated. Within each of the three field identification categories (*C. tenuimanus*, *C. cainii* or hybrid) the percentage of correct identifications was calculated from the number of marron who correctly belonged to that type group, based on their subsequent genotyping. The accuracy of field identification in relation to the genetically identified type groups was also determined by calculating the percentage of marron within each group that correctly belonged to that group. A comparison was then made between F_1 and F_2 /backcross hybrids for the level of accuracy obtained in correctly identifying these two genetic type groups as hybrids in the field.

2.2.5 Analysis of median carina length

The median carina length (MCL) (see Chapter 3, Section 3.2.3) was investigated and considered of high importance due to this morphological feature being a diagnostic character to distinguish between *C. tenuimanus* and *C. cainii*. A scatter plot of the relationship between MCL and orbital carapace length (OCL) for the four genetically identified type groups was created. This was done to investigate any clear divisions in the relationship of MCL and OCL between the genetically identified type groups.

The ratio of MCL:OCL was examined to see if it could serve as a practical field identification tool for the genetically identified type groups. A mean sample value was calculated for the marron within each genetically identified type group and statistical analysis performed to look at the overall and between-groups differences. A

nonparametric analysis of variance (ANOVA) was chosen to determine if there was an overall significant difference between the type groups, as the variances of the type groups were heterogeneous. The Kruskal-Wallis test with tied ranks, following the procedures of Zar (1999, p. 199), was used. This test calculates a corrected Kruskal-Wallis statistic (H_c) considered to be approximated by χ^2 with $k - 1$ degrees of freedom (Zar 1999, p. 198). To determine which genetically identified types groups were different from each other, a post-hoc nonparametric multiple comparison test with unequal sample sizes was used (Zar 1999, p. 225).

2.3 Results

Tissue samples were taken from 266 marron, which were then genotyped for the four marker allozyme loci. Nine marron (3.4%), spread over several sample sites, were found to be heterozygous at the *Est* locus and homozygous for the fast (F) allele at the three peptidase loci. Because Austin and Ryan (2002) found the presence of the slow (S) allele, at low frequency, at the Esterase (*Est*) locus in marron (*C. cainii*) sampled from the Blackwood River, these marron were removed from the data set because it could not be ascertained if the marron was a hybrid (most likely a backcross) or a purebred *C. cainii*. At the site where the rapid replacement of *C. tenuimanus* by *C. cainii* was recorded over a nineteen year period, the final slow (S) allele frequency for *Est* was found to be not significantly different from marron sampled from the Blackwood River in 1985 (Austin and Ryan 2002) (see Section 2.1.1). As the Blackwood River is in close proximity, directly south of the Margaret River and the actual origin of the introduced *C. cainii* is unknown, removal of these marron from the data set was deemed justified. One marron was found to be homozygous at the *Est* locus for the slow (S) allele and heterozygous at the three peptidase loci. It was sampled from a population where the slow allele had a relatively high frequency at the *Est* locus, in that this is where two of the nine marron mentioned above came from. Due to the relatively high frequency of the slow allele at the *Est* locus, it could not be ascertained whether this marron was either an F_2 /backcross hybrid or an F_1 hybrid. For this reason it was not included in any analysis that involved the separation of hybrids into genetically identified F_1 and F_2 /backcross categories. However, it was included in the analysis of field identification, as no attempt was made to distinguish between F_1 and F_2 /backcross when a marron was identified as a hybrid in the field.

2.3.1 Field identifications

The accuracy of field identifications varied between *C. tenuimanus*, *C. cainii* and hybrids (Table 2.1). All of the tissue-sampled marron identified as *C. tenuimanus* in the field had been correctly identified (Table 2.1). This shows the relative ease with which positive results are achievable in visually identifying *C. tenuimanus* in the field. This is promising for use in enabling community participation in the active removal of *C. cainii* and hybrids and in allowing recreational fishing within the distribution of *C. tenuimanus*.

Table 2.1. Accuracy of the field identification (numbers of marron) of *C. tenuimanus*, hybrids and *C. cainii*, based on morphology, in relation to the results of analysis of the four marker allozyme loci.

		Genetically Identified			Total	% Correct
		<i>C. tenuimanus</i>	Hybrid	<i>C. cainii</i>		
Field Identification	<i>C. tenuimanus</i>	66	0	0	66	100.0
	Hybrid	6	43	12	61	70.5
	<i>C. cainii</i>	0	17	113	130	86.9
	Total	72	60	125	259	
	% Correct	91.7	71.7	90.4		

Six marron genetically identified as *C. tenuimanus*, and twelve genetically identified as *C. cainii* were incorrectly identified as hybrids in the field (based on visual interpretation of their morphology) (Table 2.1). As a result, 70.5% of marron identified as hybrids in the field were correctly identified (Table 2.1). In addition, 17 (13.1%) of the 130 marron identified as *C. cainii* in the field were genetically identified as hybrids. The highest error in field identification came from F₂/backcross hybrids (Table 2.2). Just under half of this genetically identified type group was correctly identified in the field. Genetically identified *C. tenuimanus*, *C. cainii* (Table 2.1) and F₁ hybrids (Table 2.2) were all approximately 90% identified correctly as such in the field.

Table 2.2. Accuracy of field identification for genetically identified F₁ and F₂/backcross hybrids. The discrepancy in the total number of marron identified as hybrids in the field (*), in that there are 42 vs 43 in Table 2.1, is related to the marron that was found to homozygous at the *Est* locus for the fast allele and heterozygous (FS) at all three peptidase loci and not included in comparisons between F₁ and F₂/backcross hybrids.

		Genetically Identified		Total
		F ₁	F ₂ /backcross	
Field Identification	<i>C. tenuimanus</i>	0	0	0
	Hybrid	30	12	42*
	<i>C. cainii</i>	4	13	17
	Total	34	25	
	% Correct	88.2	48.0	

It should be noted that only a small selection of captured *C. tenuimanus* and *C. cainii* marron had tissue samples taken in relation to their actual proportion within populations. From this it can be predicted that the overall accuracy for genetically identified *C. tenuimanus* and *C. cainii* (Table 2.1) would have been proportionally higher if all of the marron captured from populations had tissue samples collected. This is particularly important for *C. tenuimanus* in terms of ensuring that very minimal numbers will be accidentally taken during recreational fishing or active removal

programs for *C. cainii* and hybrids. The results of this study suggest that the degree in which *C. tenuimanus* may be accidentally taken will be based on a small number of this species being incorrectly identified in the field as hybrids. No tissue sampled marron identified as *C. tenuimanus* in the field, based on morphology, was genetically identified as a hybrid or *C. cainii*. Therefore, the production of a field identification guide for use by the general public to accurately identify *C. tenuimanus* appears to be feasible, based on the results presented here.

The level of accuracy achieved in identifying, in the field, the different types of marron present, although high in all but the F₂/backcross hybrids, is not quite at the optimal 100% target. These results highlight the need for investigation of morphometric characters that may be usefully incorporated with visual features to increase the accuracy in field identification of the different marron present. Finding a strong relationship between morphometrics and morphology will be necessary if hybrids are to be identified accurately in the field to the precise level required to evaluate the extent and dynamics of hybridisation between *C. tenuimanus* and *C. cainii*.

2.3.2 Analysis of median carina length

OCLvsMCL

Significant positive correlation was found to exist between orbital carapace length (OCL) and median carina length (MCL) for genetically identified *C. tenuimanus* ($r(70) = 0.905, p < 0.001$), F₁ hybrids ($r(31) = 0.945, p < 0.001$), F₂/backcross hybrids ($r(23) = 0.713, p < 0.001$) and *C. cainii* ($r(123) = 0.913, p < 0.001$) (Figure 2.1).

The weaker correlation of the F₂/backcross hybrids, although still significant, is reflected in the poor accuracy obtained in correctly identifying these marron as 'hybrids' in the field, based on their morphology (Table 2.2). This highlights a variable morphology associated with this genetically diverse group (see Box 2.1).

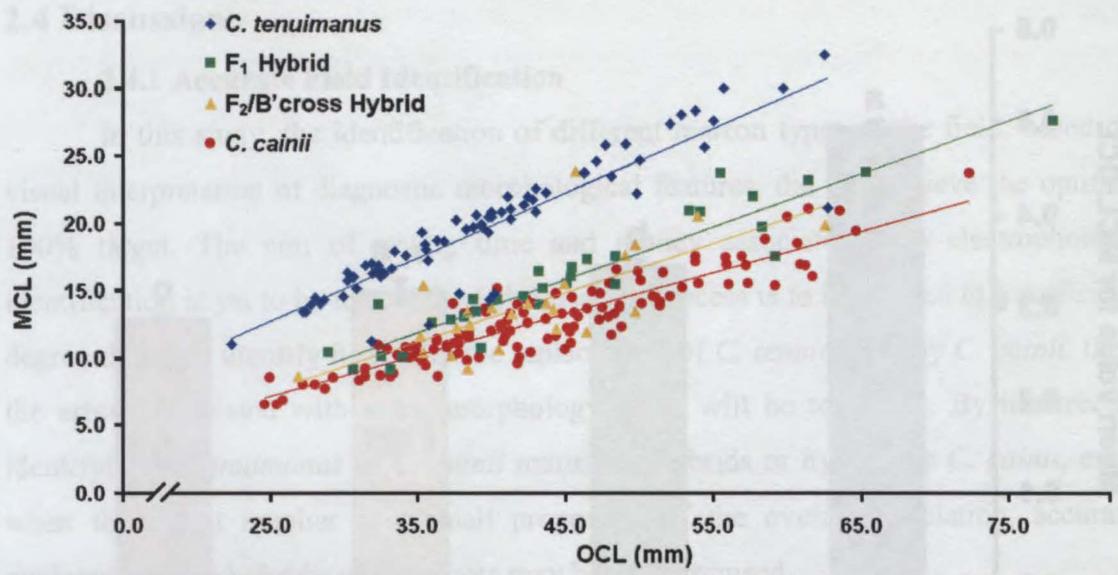


Figure 2.1. Orbital carapace length (OCL) and median carina length (MCL) relationship for the genetically identified type groups.

The *C. tenuimanus* data points that sit amongst the *C. cainii* and hybrid (both types) data points in Figure 2.1, represent marron that were incorrectly identified in the field as hybrids. It is likely that the median carina of these marron thought to be hybrids did not appear continually uniformly raised, consequentially was not measured to the cervical groove, and hence its position in relation to OCL (Figure 2.1). The *F₂/backcross* data point that sits just above the *C. tenuimanus* trend line is a good example of the morphological variation of this group. This marron is pictured in Box 2.1 (b) and was photographed due to its unusual morphology. It had a clear continuous median carina, hence its association with the *C. tenuimanus* data points in Figure 2.1, but the carapace lacked distinctive clusters of setae. It is assumed that this marron is the result of a hybrid backcross with *C. tenuimanus*, due to its morphology and genotype at the four allozyme marker loci (Box 2.1).

MCL:OCL Ratio

An overall significant difference was found between the mean values of the MCL:OCL ratio for the four genetically identified type groups (Figure 2.2) following the nonparametric Kruskal-Wallis test with tied ranks ($H_c = 170.3, df = 3, p < 0.0001$).

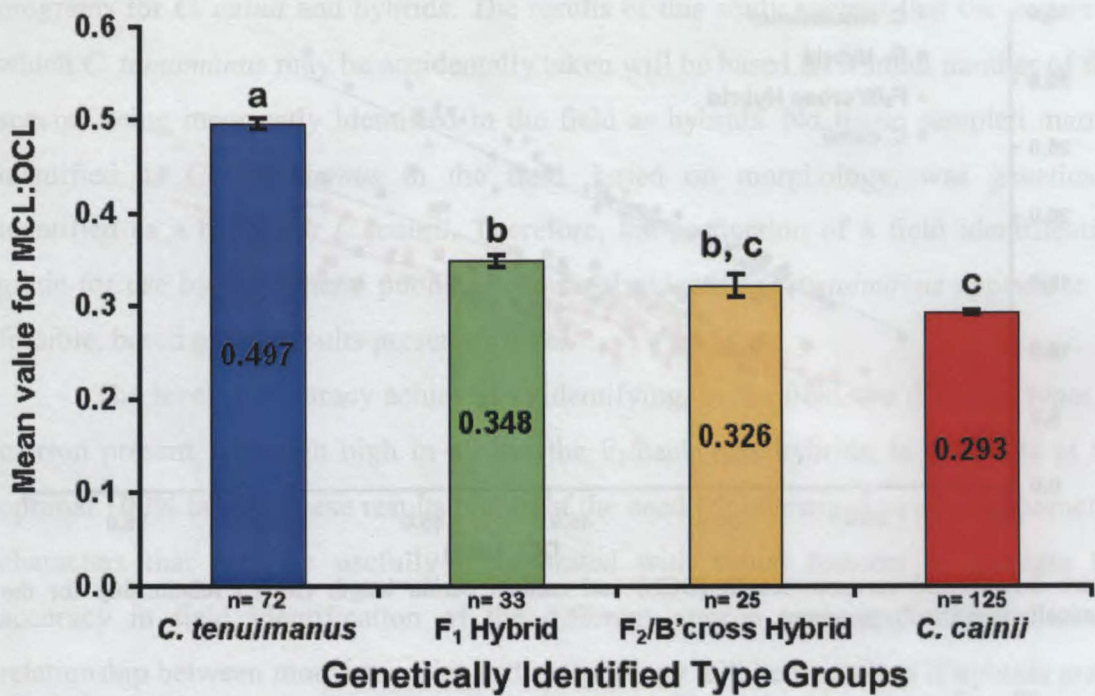


Figure 2.2. Differences in the mean ratio of MCL:OCL (\pm S.E.) between the genetically identified type groups. Values are expressed within bars. The letters (a, b or c) above the groups represent the results of the post-hoc multiple comparison test (Table2.1). Groups with matching letters were not significantly different from each other.

This result suggests that the MCL:OCL ratio has the potential to be used as a tool in the identification of hybrids in the field, given that MCL can be correctly measured. However, the post-hoc nonparametric multiple comparison test revealed that the mean MCL:OCL ratio for the F₂/backcross hybrids was not significantly different from that of the F₁ hybrids ($Q = 1.63, k = 4, p > 0.5$) or *C. cainii* ($Q = 2.33, k = 4, p > 0.1$) (Table 2.3). All other comparisons were significantly different from each other (Table 2.3).

Table 2.3. Nonparametric multiple comparison test of mean MCL:OCL ratio for the genetically identified type groups using pairwise difference of mean ranks. Critical value for this test given as $Q_{\alpha, k..}$ groups marked with an asterix (*) were significantly different.

Comparison	Difference	S.E.	Q	$Q_{0.05, 4}$	p-value
<i>C. cainii</i> vs <i>C. tenuimanus</i> *	140.113	10.82	12.95	2.639	< 0.001
<i>C. cainii</i> vs F ₁ Hybrid*	69.737	14.29	4.88	2.639	< 0.001
<i>C. cainii</i> vs F ₂ /Backcross Hybrid	37.191	16.27	2.33	2.639	> 0.1
F ₁ Hybrid vs <i>C. tenuimanus</i> *	102.194	17.18	5.95	2.639	< 0.001
F ₁ Hybrid vs F ₂ /Backcross Hybrid	31.818	19.55	1.63	2.639	> 0.5
F ₂ /Backcross Hybrid vs <i>C. tenuimanus</i> *	70.376	15.32	4.59	2.639	< 0.001

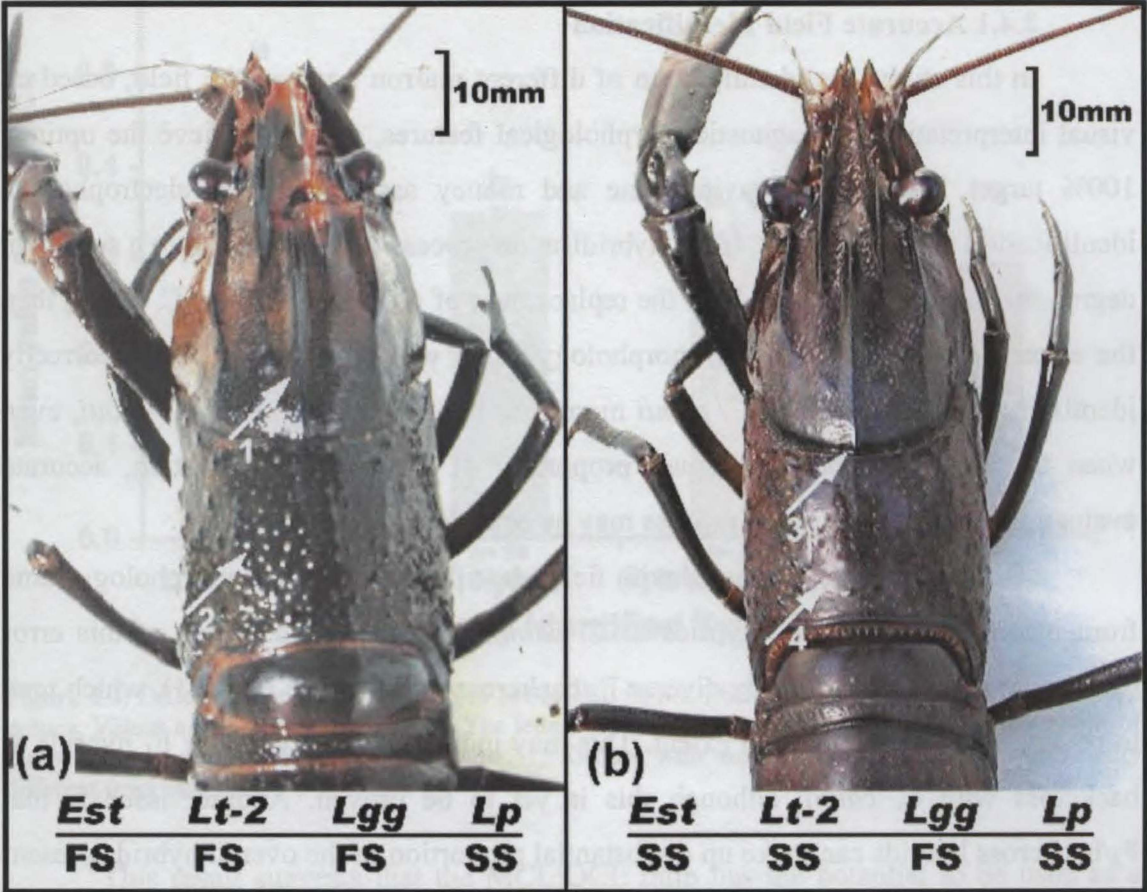
2.4 Discussion

2.4.1 Accurate Field Identification

In this study, the identification of different marron types in the field, based on visual interpretation of diagnostic morphological features, did not achieve the optimal 100% target. The aim of saving time and money associated with electrophoretic identification is yet to be met. If the hybridisation process is to be studied to a sufficient degree that may identify its role in the replacement of *C. tenuimanus* by *C. cainii*, then the errors associated with using morphology alone will be too great. By incorrectly identifying *C. tenuimanus* or *C. cainii* marron as hybrids or hybrids as *C. cainii*, even when the actual number is a small proportion of the overall population, accurate evaluation of the hybridisation process may be compromised.

Most of the error associated with field identification based on morphology came from incorrectly identifying hybrids as *C. cainii*, and *vice versa*. Much of this error came from the morphologically diverse F₂/backcross hybrids (see Box 2.1), which tend to resemble *C. cainii* to a high extent. This may indicate a preference for F₁ hybrids to backcross with *C. cainii*, although this is yet to be proven. Another issue is that F₂/backcross hybrids can make up a substantial proportion of the overall hybrids present in sympatric populations. The problem with field identification of marron is also compounded by the occasional incorrect identification of *C. cainii* (see Box 2.2) and to a lesser extent *C. tenuimanus* as hybrids. These errors associated with *C. cainii* and *C. tenuimanus* could be an indication of such factors as size variations for morphological characters. This could further reduce the ability to evaluate the hybridisation process through the inaccurate identification of marron types in the field.

Box 2.1



Two F_2 /backcross hybrids showing variation in morphology for this genetically identified type group.

(a) This marron (OCL=53.9 mm) has morphological features one would associate with a typical F_1 hybrid. It has a median carina that is not completely continuous to the cervical groove (arrow 1) and has stubble like setation (arrow 2). The genotype of this marron for the four marker loci places it as a possible F_2 hybrid.

(b) This marron (OCL=45.7 mm) has a continuous median carina (arrow 3) typical of *C. tenuimanus*, but lacks the distinctive clusters of setae (arrow 4). Based on the genotype, and morphology, this marron is a likely offspring from a hybrid backcross with *C. tenuimanus*.

It is clear that if the required target of 100 % accuracy in identification of hybrid marron is to be met to negate the need for electrophoretic analysis, then morphology alone will not suffice. The use of morphometric characters could be considered highly important. The examination of median carina length (MCL) carried out in this study has proved to be a good starting point but is evidently not a morphometric feature that could be used on its own. The relationship between orbital carapace length (OCL) and MCL was a good indicator in separating *C. tenuimanus* from the other genetically identified type groups. There was, however, too much overlapping variation between the other three genetically identified type groups. The F_2 /backcross hybrids had the greatest variation, due to the differences in morphology associated with this group. This may be problematic when trying to distinguish this genetic type group from the others. From a

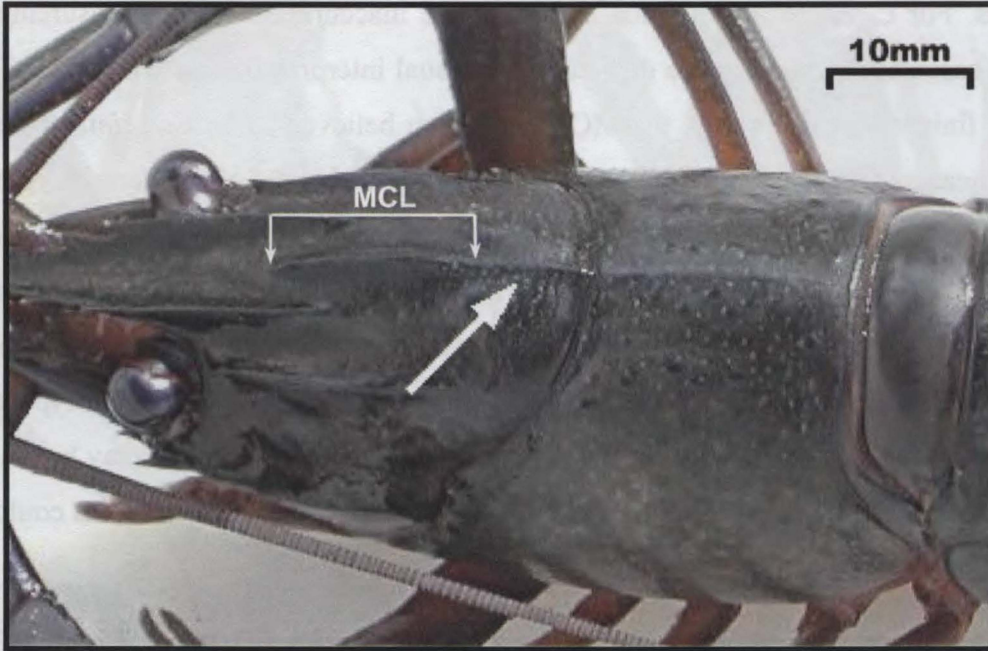
practical field identification viewpoint, the difficulties that may arise from inaccurate measurement of MCL would add to this problem. For *C. tenuimanus*, with a median carina that is meant to be continuous to the cervical groove, there should not be any difficulty associated with measurement of MCL once a marron is believed to be this species. For *C. cainii* and hybrids there may be inaccuracies in the measurement of MCL, associated with possible difficulties in visual interpretation of where the median carina finishes. In this study, the MCL of marron believed to be *C. cainii* or hybrids were measured from where the median carina began anteriorly to the terminal point of inflection from the prominently raised section, where there may have been (mostly hybrids) or may not have been a weakly raised section that continued to the cervical groove, or became conspicuous again just prior to. Small errors either way could pose a problem in using the MCL to OCL relationship to find diagnostic genetic type group differences. Characteristic differences between the genetic type groups may be small to begin with, which will make it more difficult to distinguish between them, as could size related development of the median carina.

Although the MCL:OCL ratio had an overall significant difference for the four genetically identified type groups, this was not found to be the case for all comparisons between groups. Also, the overlapping OCL and MCL relationship for the genetically identified type groups (Figure 2.1) is not represented in mean MCL:OCL values (Figure 2.2) of these groups. This overlapping variation suggests that the MCL:OCL ratio is not sufficiently useful as an identification tool on its own. An overlap in diagnostic morphometric features will not be appropriate for correct identification of hybrid marron in the field. All forms of hybrids and *C. tenuimanus* and *C. cainii* will need to be clearly distinguished if the hybridisation process is to be evaluated in populations. The MCL:OCL ratio may be a better identification tool if used in conjunction with other morphological and/or morphometric characters. A "hair" index based on the level of setation could be one such associated feature, but will need to be linked with size, due to likely developmental differences. However, setation might only serve to identify *C. tenuimanus*, as it may not be useful in distinguishing between *C. cainii* and hybrids. A complete lack of setae could be a direct indicator of *C. cainii*, although, setation tended to be a characteristic that was not largely associated with hybrids. Even small juvenile *C. tenuimanus* tended not be overly setose (pers. obs.).

Further investigation into an association between morphometric and morphological characters, and size relationships, will be required if more accurate diagnostic identification tools are to be developed. The margin of error connected to

such identification tools will need to be set at a practical level, related to correctly identifying all marron types present, so the degree of hybridisation in populations can be accurately measured.

Box 2.2



A genetically identified *C. cainii* (OCL=41.1mm) that was identified in the field as a hybrid. The arrow indicates the post median carina ridge, compared to the actual MCL measured, which would have led to the belief that this marron was a hybrid. This weakly raised ridge can be present in small sized *C. cainii* and is likely related to development, as it is mostly not present in larger, older marron. The MCL:OCL ratio for this marron was 0.309, which is very close to mean value for *C. cainii* of 0.293. Using this ratio in the field may have resulted in correct field identification.

Perry *et al* (2001b) undertook a detailed morphometric analysis between the North American freshwater crayfish species, *Orconectes rusticus* and *O. propinquus*, and their hybrids. Twelve morphometric characters were compared between allopatric populations, with the two species being successfully separated on the basis of the relationship between two ratios involving five characters. However, when this relationship was tested against sympatric populations for genetically identified *O. rusticus*, *O. propinquus*, F₁ hybrids and F₂/backcross hybrids, the hybrid types did not separate into distinct groups. Although not the aim of Perry *et al*'s (2001b) study, it shows that even with the aid of numerous morphometric characters the clear separation of hybrid marron from pure types, and the separation of F₁'s from F₂/backcrosses, will be difficult to achieve in the field. The discovery of F₂ or backcross hybrids derived from *O. rusticus* and *O. propinquus* is believed to have been possible only through the use of molecular markers and not possible using morphology (Perry *et al*. 2002).

An important consideration in developing morphometric and morphological associations as a field identification tool will be the practical field use. Several small-scale measurements that require calculations to produce the appropriate relationships may not be suitable for field identification. In the present study it took approximately two minutes to process each marron in the field. This involved making a species identification in the field and recording basic morphometric features (see Chapter 3). It could take much longer if other morphometric characters are required to be measured, calculations carried out and associations determined. Many hours may be needed to process all the marron captured during a single night of trapping. If 'experts' are in short supply, catching effort may need to be reduced and extended field trips might be required, possibly beyond a practical time frame. If an acceptably accurate field identification tool could be developed, based on morphology and/or morphometrics, then the potential added time and cost of field work would have to be weighed against the time and cost associated with genetic analysis in a laboratory.

Even if F_1 and F_2 /backcross hybrids are found to be accurately identifiable in the field, the information this would provide on hybridisation may be limited. The parental origins of the F_1 and F_2 /backcross hybrids are also needed to help in the evaluation of the hybridisation process. Perry *et al* (2001a) found, following mtDNA analysis, that 94.5% of their F_1 hybrids came from matings between *O. rusticus* females and *O. propinquus* males. This kind of data is not obtainable from the allozyme marker analysis used for marron, yet is extremely informative. It would not be suitable for distinguishing between F_2 and backcross hybrids, which is needed to determine the actual hybridisation processes occurring. Other genetic studies can be carried out on tissue samples that can provide a greater insight than the identification of hybrids using allozyme markers alone. If time and money prove not to be a limited resource, then the collection of tissue samples is recommended.

2.4.2 Distinguishing the Margaret River Hairy Marron

The results of the present study show that *C. tenuimanus* can be readily distinguished from *C. cainii* and hybrids in the field based on morphology. Therefore, it is possible to produce a basic identification guide for use by members of the general public to enable them to participate in active removal programs. Such an identification guide would also be extremely valuable during the recreational fishing season for marron within the Margaret River. Identification guides could be issued when a recreational fishing licence is purchased to ensure that all people fishing for marron in

the Margaret River know how to identify *C. tenuimanus*. During the 2003 and 2004 recreational marron fishing seasons, fishing was banned in the Margaret River upstream of the intersection with Ten Mile Brook (see Chapter 3, site 7) as a means of protecting the remaining populations of *C. tenuimanus*. However, recreational fishing could be used as a way of increasing the recruitment ability of *C. tenuimanus* by decreasing the amount of *C. cainii* present in sympatric populations. This, of course, relies on people being able to identify accurately and avoid taking *C. tenuimanus* and knowing that specimens of this species are not to be taken.

It should be possible to incorporate the intermediate features of at least F_1 hybrids into identification guides for use by the general public. However, correctly distinguishing hybrids from *C. cainii* will not be necessary, as both will need to be removed to protect *C. tenuimanus*. It will, however, be necessary to ensure that as few specimens as possible of *C. tenuimanus* are mistakenly identified as hybrids and accidentally removed. Based on Table 2.1, it could be estimated that only 10% of the marron in a population that are identified in the field as hybrids are in fact *C. tenuimanus*. As hybrids generally comprise a small proportion of marron present in populations (see Chapter 3) it can be reasonably expected that very few specimens of *C. tenuimanus* would be misidentified in this way. Based on this 1-in-10 field identified hybrids being in fact *C. tenuimanus*, and if there is ratio of about 4:1 *C. tenuimanus* to hybrids in a typical sympatric population (see Chapter 3), then it could be estimated that only 2.5% of the *C. tenuimanus* population might be misidentified as hybrids and accidentally removed.

Although it has been recognized in this study that with relative novel expertise, *C. tenuimanus* can be readily distinguished from *C. cainii* and hybrids, the possibility of error in identification must still be considered. This would most likely be due to a hybrid marron being misidentified as *C. tenuimanus*. This will be of concern when a captive breeding population of *C. tenuimanus* is established from marron captured from the wild, for instance as part of a recovery plan involving active restocking of the Margaret River with *C. tenuimanus* produced from this captive population. Ensuring that this captive population does not contain *C. cainii* genes is going to be crucial in guaranteeing that the progeny produced for restocking are genetically uncontaminated. The current captive breeding stock of *C. tenuimanus* held by the Western Australian Department of Fisheries is subject to this concern. Marron believed to be *C. tenuimanus* that are taken from the wild for such captive breeding populations should, at the least, be screened for the four marker allozyme loci.

2.4.3 Conclusions

C. tenuimanus has been shown to be readily distinguished in the field from both *C. cainii* and hybrids on the basis of visible morphological features. The production of a practical field identification guide for use by members of the general public to accurately identify *C. tenuimanus* is feasible. Such a field identification guide would serve to educate people on the plight of the Margaret River hairy marron. It would give the opportunity for community participation in active removal programs and allow recreational fishing to continue within the known distribution of *C. tenuimanus*. Accidental removal of *C. tenuimanus* due to misidentification should be minimal and not of great concern. A prototype of a practical and informative field identification guide, for use by members of the general public, has been constructed (Figure 2.3) and can be scrutinised before possible publication.

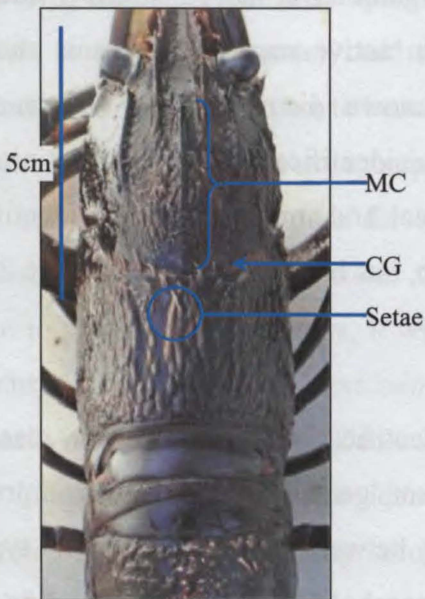
At this stage, the production of a field identification guide for use by researchers and managers to distinguish between the different 'genetic' forms of marron present is not feasible. Being able to distinguish accurately between the different hybrid types and *C. cainii* poses the greatest challenge. Further morphological and morphometric studies will be required. However, even if advances are made, the practicality of a detailed field identification guide is in question. Therefore, the ability to identify correctly the genetic forms of marron in the field at the optimal level required for evaluation of the hybridisation process remains in doubt. The taking of tissue samples for analysis of the four allozyme marker loci is still required at present. However, tissue sample analysis will provide the opportunity for more complex genetic analysis involving DNA that would ultimately provide a more accurate insight into the hybridisation process occurring within sympatric populations.

Identification Guide for Marron Within the Margaret River

Certain physical characteristics can be used to distinguish the critically endangered Margaret River hairy marron (*Cherax tenuimanus*) from the introduced smooth marron (*Cherax cainii*). Below is an identification guide for marron within the Margaret River to ensure 'hairy' marron are not removed.

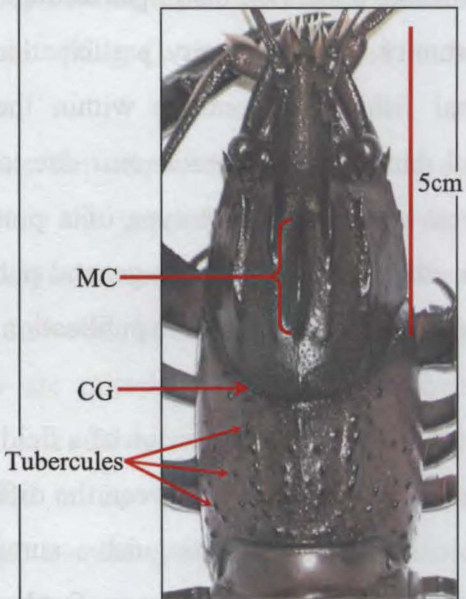
Setation ('Hairs')

Margaret River 'hairy' marron have clusters of setae (hairs) that cover their carapace and tail. The degree of setation will depend on size with larger individuals being more setose ('hairy').



Margaret River 'Hairy' Marron

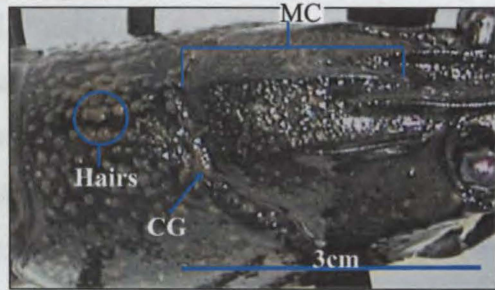
Introduced 'smooth' marron do not have setae on their carapace or tail. This species also has tubercules (bumps) on their carapace and tail that are much less pronounced in 'hairy' marron.



Introduced 'Smooth' Marron

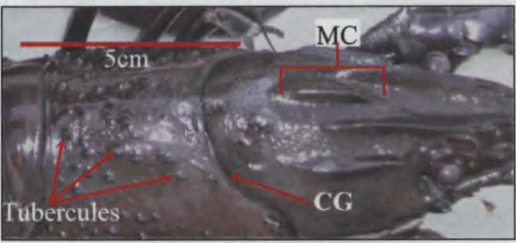
Median Carina (Central Ridge)

The median carina (MC) is the central ridge on the carapace of marron. In 'hairy' marron the MC is not strongly raised and is continuous to the cervical groove (CG).

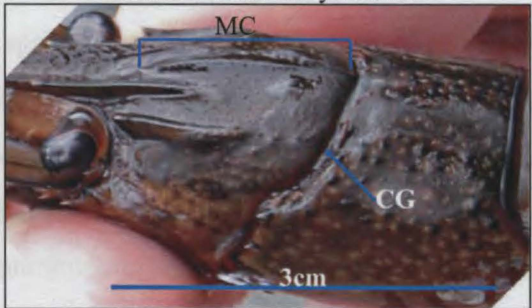


Medium sized 'hairy' marron

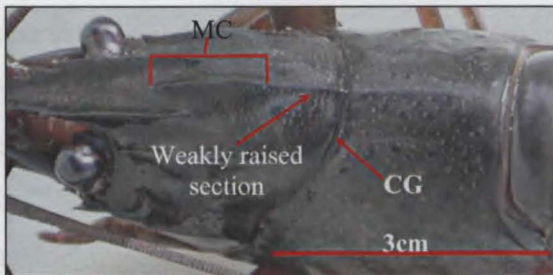
The MC in 'smooth' marron is much more protrusive and is considered not to be continuous to the CG. There can be a weakly raised section that follows, which does extend to the CG.



Large sized 'smooth' marron



Small sized 'hairy' marron



Medium sized 'smooth' marron

Figure 2.3. Prototype of field identification guide aimed at members of the general public to provide them with the ability to identify *C. tenuimanus* correctly.

Chapter 3: Aspects affecting the distribution of marron species within the Margaret River



Various sites along the length of the Margaret River from the lower reaches at the top right and then clockwise to the upper reaches at the top left. The central image is Cane Break Pool, located in State forest in the upper reaches. This is where the mark-recapture study was undertaken.

3.1 Introduction

3.1.1 Mapping the distribution of marron within the Margaret River

Monitoring of marron within the Margaret River since the introduction of *C. cainii* sometime in the early 1980s has been neither regular nor broad-scale (Austin and Ryan 2002, Molony and Bird 2002). Although the date of the introduction of *C. cainii* can be estimated, the dispersal of this species within the Margaret River and the rate at which it has replaced *C. tenuimanus* remain unclear. There are a small number of sites within the Margaret River that have been monitored repeatedly, but at irregular intervals. Austin and Ryan (2002) detail the repeated sampling of a single site a short distance upstream of the Margaret River townsite. It was at this site that *C. cainii* was first discovered in the Margaret River. This was followed by a nineteen year period in which its dramatic replacement ability over *C. tenuimanus* was documented (Austin and Ryan 2002). A detailed distribution map of the different marron species within the Margaret River has not yet been constructed. The location of the remaining populations of *C. tenuimanus* has not been known in its entirety, apart from a small number of sites around, and including, Cane Break Pool in the uppermost reaches. The full distribution of *C. tenuimanus*, and the rate in which *C. cainii* has invaded and increased in proportion in the upper reaches is unknown. It was recommended by Austin and Ryan (2002) that a thorough survey of the Margaret River be undertaken to locate the remaining populations of *C. tenuimanus*.

Information needed for the conservation of a threatened species is knowledge of where it occurs and its abundance within this remaining distribution. In the case of *C. tenuimanus* it will be important to know also the location and abundance of *C. cainii*, as will the relative proportions of the two species where they exist in sympatry. This equally applies to gaining an understanding of potential extrinsic factors that could be related to the distribution and relative proportions of both marron species within the Margaret River, and hence help in determining some of the replacement mechanisms operating.

3.1.2 Mark-recapture study of a sympatric population

One particular site that is known to contain both species of marron is Cane Break Pool, located about 20 km north east of the Margaret River townsite within state forest. This pool was sampled in 1998, by Austin and Ryan (2002) who found only 17% of their catch to be *C. tenuimanus*. The Western Australian Department of Fisheries (DoF) claim approximately 60% of the marron at Cane Break Pool were *C. tenuimanus*

in 1996 (Molony and Bird 2002). This location provides a very good opportunity to investigate intrinsic properties of *C. cainii* and *C. tenuimanus*. Direct comparisons between the two species can be made within the same environment. This information will significantly assist in evaluating the potential replacement mechanisms operating.

A repeated mark-recapture study should be a useful method to compare possible differences in intrinsic factors between *C. tenuimanus* and *C. cainii*. Growth rates for marron can be estimated from an increase in body size from when they were originally caught and marked to when they were recaptured. A species mean can be generated that will provide a comparison between *C. cainii* and *C. tenuimanus*. It could be hypothesised that *C. cainii* has a greater growth rate than *C. tenuimanus*, which contributes to replacement based on the mechanisms outlined in Chapter 1. Movement patterns can be determined from distances travelled and the interval between marking and recapture (Robinson *et al.* 2000). The hypothesis here could be that *C. tenuimanus* exhibits a greater degree of movement due to competitive exclusion from *C. cainii*.

Repeated sampling from the same location is another means of estimating growth rates. The clustering of individuals in size-frequency histograms can identify individual cohorts (King 1995). These modal peaks can be followed over repeated sampling and an estimate of growth rate for the particular cohort can be calculated (King 1995). Once again it could be hypothesised that *C. cainii* would have a greater estimated growth rate than *C. tenuimanus*. The recruitment of new cohorts into the catchable size range can also be detected in repeated sampling by constructing size-frequency histograms (King 1995). An earlier recruitment by *C. cainii* into the catchable size range would be hypothesised, indicating potentially a greater growth rate and/or greater competitive ability for food sources.

Sampling around the spawning period could provide insight into differences in reproductive timing between the two species. Comparisons in the stage of offspring development and activity of females might indicate the timing of brood release. *C. cainii* would be hypothesised to have an earlier spawning period brood release than *C. tenuimanus*, related to an advantage for *C. cainii* YOY recruitment (see Chapter 1).

By conducting a mark-recapture study at Cane Break Pool, an attempt was made to establish differences in intrinsic factors between *C. cainii* and *C. tenuimanus* based on the testing of proposed hypotheses. This study will provide a starting point for directing appropriate action plans based on countering specific replacement mechanisms. A basis for continued population monitoring at Cane Break Pool, to assess

potential conservation actions aimed at preventing the extinction of *C. tenuimanus*, will also be established.

3.1.3 Aims

Marron distribution map

A distribution survey was conducted in the Margaret River for the different marron species and involved mapping the current remaining populations of *C. tenuimanus* along with that of the introduced *C. cainii*. The relative proportion of *C. cainii*, *C. tenuimanus* and hybrids, within populations were measured. This distribution map will serve to:

1. Highlight areas that can be targeted for conservation efforts.
2. Help establish if the replacement of *C. tenuimanus* by *C. cainii* in different locations is influenced by extrinsic factors associated with these locations.
3. Provide a basis for a continued monitoring program of *C. tenuimanus* within the Margaret River.

Cane Break Pool mark-recapture study

A repeated mark-recapture study was undertaken in a known sympatric population within the Margaret River, Cane Break Pool. Potential differences in intrinsic factors between *C. tenuimanus* and *C. cainii* were explored. Based on these, evidence was sought to test the following hypotheses:

1. *C. cainii* has a faster growth rate than *C. tenuimanus*.
2. *C. tenuimanus* has a greater degree of movement around Cane Break Pool.
3. *C. cainii* has an earlier spawning period and brood release than *C. tenuimanus*.
4. *C. cainii* has an earlier recruitment of individuals into catchable size.

Once evaluated, a foundation for potential management strategies can be achieved, based on countering the negative effects that differences in intrinsic factors have on the replacement of *C. tenuimanus* within the Margaret River. The basis for continued monitoring of this location can also be established.

3.2 Methods

3.2.1 Site Selection

Distribution survey

The Margaret River is a temporary flowing system that is reduced in late summer to a series of permanent pools that vary in size and depth throughout its length. Sampling was undertaken from January 2002 to May 2003 during all seasons. Permanent pools were selected, as they could be expected to have permanent populations of marron. These pools were identified from 1:25000 topographic maps and verified through the use of online digital high-resolution aerial photographs viewed on SkyView WA (<http://www.landonline.com.au>) from the Western Australian Department of Land Information (DLI). The aerial photographs were dated March 2001, meaning they gave indication of permanent pools, as this is the time of year that water levels are at their lowest in the Margaret River. Ease of access determined which particular pools were to be sampled, and selection was carried out in the field. Twenty-one sites were sampled, ranging from the uppermost pool in state forest to a small pool slightly upstream of the estuarine section at the mouth of the river. This included sampling a large storage dam situated on a tributary of the Margaret River, Ten Mile Brook Dam, the townsite's water supply.

Mark-recapture study

Cane Break Pool was chosen for the repeated mark-recapture study because of its sympatric population of *C. tenuimanus* and *C. cainii*. Cane Break Pool is an official camping ground managed by the Western Australian Department of Conservation and Land Management (DCLM) and has year round access by road, which made it an ideal location for repeated sampling events. Water visibility is clear year round and there are numerous areas suitable for all methods of capture. This site is also suitable for the use of a canoe, which can be launched with relative ease.

3.2.2 Equipment Type and Usage

Baited traps

The traps used were 'opera house' style folding traps (Figure 3.1) with nylon hauling rope purchased from fishing and sporting stores. Two different sizes were used, a standard size (65x50 cm base) and a "jumbo" size (90x60 cm base), each with 10x10 mm nylon netting lining. Traps were baited with supermarket purchased chicken feed enclosed within a single layer of nylon stocking. Placement of traps at study sites was

generally undertaken before sunset from the edge of banks or from the side of a canoe within the pool itself. Undertaking the placement of traps at this time was important in order to maintain visibility during the process to enable the avoidance of snags (e.g. submerged logs), and to ensure the lie of the traps was not compromised so as to hinder the entering of marron. Once traps were placed in the water they were tied to suitable objects such as riparian vegetation and large woody debris or, when a canoe was utilised, the nylon hauling rope was attached to a float.

The positioning of traps in relation to each other varied from being competitive (overlap of area of attractiveness) to non-competitive (Morrissey 1975a). This was dependent on the suitability of positions within study sites, based on the need to place all traps in a location that was free from snags or the need to spread out traps in order to cover as much of a study site as possible.

The baited traps were mostly left in position overnight and not hauled until the following morning. On several occasions, however, traps were hauled after several hours of being placed in the water due to the intention of not returning to the site the following morning.

Traps were the favoured method of capture as they could be placed and left overnight, allowing daylight hours for lengthy processing. It was possible to leave captured marron in traps until all the marron from another trap had been processed and returned to the water. This had the advantage of keeping marron in the water thereby reducing stress on individuals. Any association with the species of marron captured and the location of the trap could also be explored.

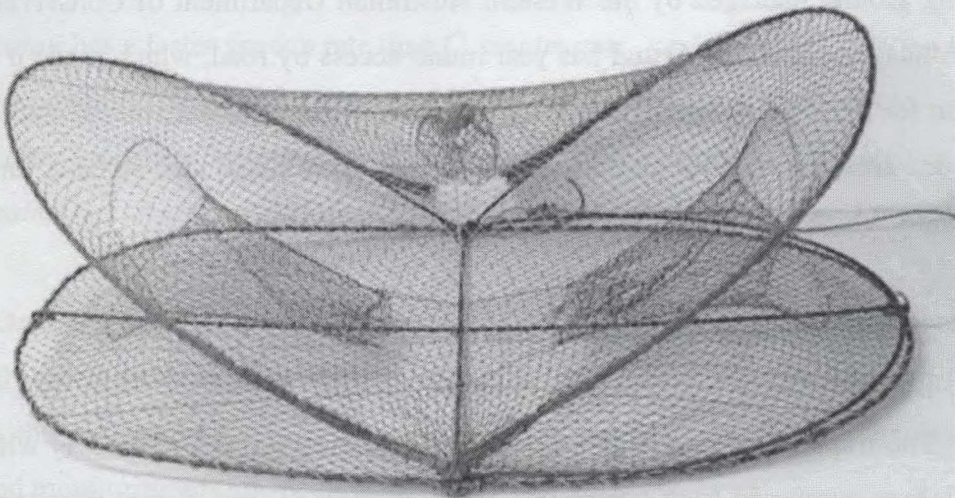


Figure 3.1. 'Opera house' style folding trap used to capture marron. Jumbo size (90x60 cm base) pictured.

Baited drop nets

Drop nets used in this study consisted of modified standard marron drop nets and custom made drop nets obtained from the Fisheries Department of Western Australia. The standard marron nets are obtainable from most fishing stores in the south west of Western Australia and are the only type permitted to be used during the recreational marron fishing season. These drop nets have a 60 cm diameter top steel ring and a 50 cm diameter bottom steel ring, which are joined together by 3x3 cm nylon netting. They have 32x85 mm steel mesh base designed to prevent the capture of marron under the minimum legal size of 76 mm RCL (Morrissey 1989). Several metres of 4 mm nylon hauling rope are attached to the top steel ring. The modifications consisted of lining the steel base with 10x10 mm plastic "handy" mesh, available from most hardware stores and lining the middle with 10x10 mm nylon netting, which then allowed the capture of small sized marron. The custom made drop nets from the Fisheries Department of Western Australia have a 60 cm diameter top steel ring and a 50 cm diameter bottom steel ring. Both the lining and the base are fine mesh nylon netting and a thick nylon hauling rope with a hooped end was attached to the top steel ring. A plastic bait tray was attached centrally to the nylon netting base. The drop nets were baited with chicken pellet filled stockings as used for the traps, which attached centrally to both types of drop net.

Drop nets were positioned at places where rapid hauling was not compromised, which is necessary to minimize the escape of marron during the capture process. Places free from snags were also a necessity to prevent drop nets from tangling. Finding such locations at sites often proved difficult. As marron were to be returned to their place of capture and required a time-consuming processing period, longer than desired hauling intervals were often required. Captured marron from one hauling event would need to be processed and returned to the water before another could be undertaken. That procedure significantly reduces the catch per drop net over the sampling period compared to traps, where there is no issue of processing marron between hauls. Because of the inability to utilise drop nets to their full potential (Morrissey 1975b, 1996) they were not the favoured method of capture for this present study.

Scoop net

An aluminium frame scoop net was used, which had a triangular end piece with a 40 cm leading edge and 10x10 mm nylon netting. A bait station or bait line, using chicken pellets, was set up at the edge of the riverbank just before or just after nightfall

and then checked at regular intervals. An attempt was made to capture marron that had been attracted to the chicken pellets. Bait stations consisted of one to two handfuls of chicken pellets placed in a single location, such as off the edge of rocks or at locations on the riverbank where there was limited access to the water for the wide range use of a scoop net. Bait lines consisted of spreading a thin line of chicken pellets along the edge of riverbanks in areas with wider access to the water to allow the use of a scoop net.

The scoop net was sometimes used as an opportunistic capture method during periods of night time drop netting or when traps were hauled during mornings. Occasionally, marron that had been attracted to the bait but had not entered the capture device were situated adjacent to drop nets or traps. An attempt was made to capture such marron using the scoop net.

Basic water measurements

Water measurements were taken at sampling sites mostly from the edge of the riverbank or rocky platforms at a depth between 50 and 100 cm. On occasion measurements were taken from the side of a canoe if one was being used to place traps at a particular sampling site. This measuring was generally undertaken after all captured marron had been processed and returned to the water.

The pH was measured to two decimal places using a CyberScan pH 100 hand-held meter (Eutech Instruments). Dissolved oxygen was measured in mg/L to one decimal place using an Orion Model 840 DO meter. Conductivity was measured as $\mu\text{S}/\text{cm}^2$ using a CyberScan Con 100 hand-held meter (Eutech Instruments). The dissolved oxygen meter was used to measure temperature as $^{\circ}\text{C}$ to one decimal place.

3.2.3 Processing of Captured Marron

Measurement of morphometric features

Mitutoyo stainless steel vernier callipers were used to take measurements to the nearest ± 0.1 mm. The following morphometric features were recorded from captured marron: rostral carapace length (RCL), orbital carapace length (OCL), median carina length (MCL), propodus (claw) length (PL) and propodus width (PW). A body plan of a marron showing the positioning of measurements is given in Figure 3.2. The median carina length has been treated in this study as a morphometric character (and multistate, see below), where it has previously only been treated as a multistate character (Austin and Knott 1996). The MCL for *C. cainii*, and hybrids, was measured from the anterior margin of the MC to the terminal point of inflection from the prominently raised section

where there may (mostly hybrids) or may not be a weakly raised section that continues to the cervical groove or becomes conspicuous again just prior. In *C. tenuimanus*, MCL is measured from the anterior margin to the intersection with the cervical groove, which is where the terminal point of inflection is for the continuous MC.

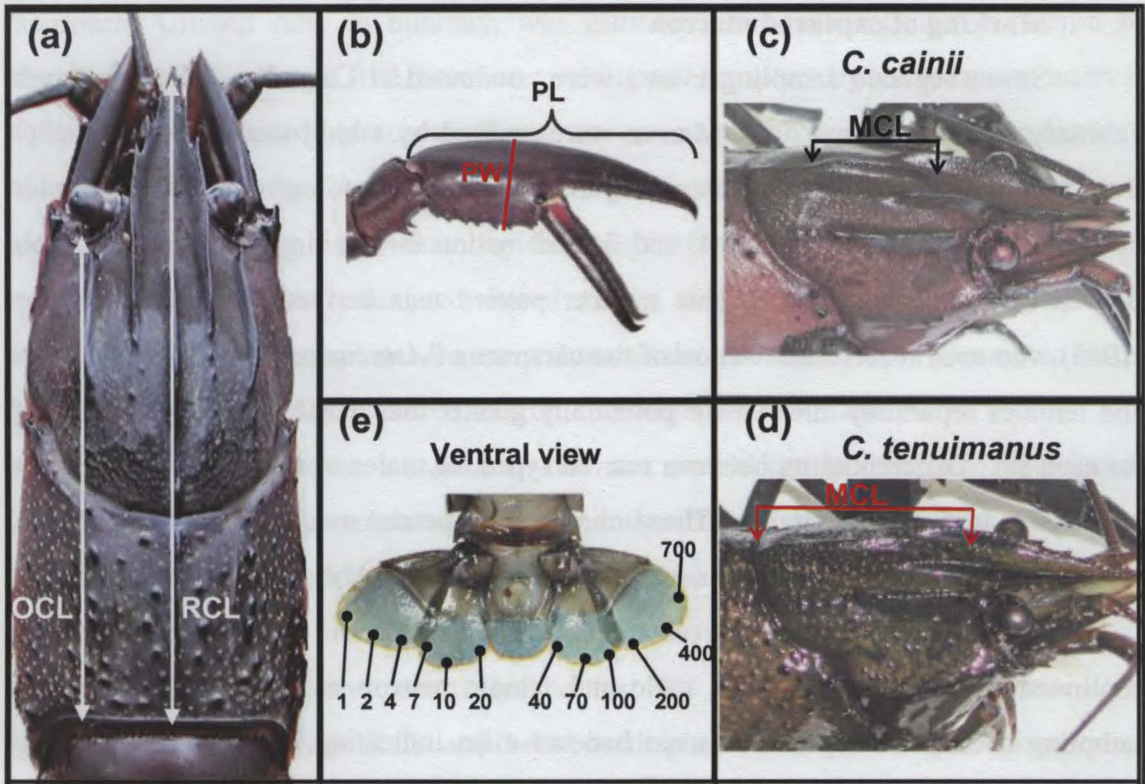


Figure 3.2. Measurements taken from captured marron. (a) Orbital carapace length (OCL) is taken from the posterior edge of the eye socket to the posterior margin of the carapace. The rostral carapace length (RCL) is from the anterior tip of the rostrum to the posterior margin of the carapace. (b) Propodus (claw) length (PL) is taken from the anterior tip to the posterior articulation point. Propodus width (PW) is measured laterally. (c) Median carina length (MCL) for *C. cainii*, and hybrids, is measured from the anterior margin of the MC to terminal point of inflection from the prominently raised section where there may (mostly hybrids) or may not be a weakly raised section that continues to the cervical groove or becomes conspicuous again just prior. (d) MCL for *C. tenuimanus* is measured from the anterior margin to the intersection of the MC and cervical groove, which is where the terminal point of inflection is for the continuous MC. (e) The tail clips for the mark-recapture study at Cane Break Pool followed a 1 2 4 7 number pattern on the ventral side, which would have allowed over 1000 marron of each sex to be uniquely numbered.

Morphological features and species identification

The median carina was treated as a multistate character, in addition to a morphometric character, and recorded as being long (continuous to the cervical groove), short or medium. The presence or absence of diagnostic setae on the carapace of marron was recorded. A general condition of the captured marron was recorded and included information on missing appendages, regenerating appendages, conspicuous smaller claw(s), general body damage and unusual morphological features, for example. Any female that was carrying developing eggs or juveniles was recorded; as was an estimate of the number she was carrying. Marron were recorded as *C. tenuimanus*, *C. cainii* or

hybrids, using the morphological features identified and following the principles outlined in Section 2.2.1.

3.2.4 Mark-Recapture Study at Cane Break Pool

Marking of captured marron

Seven separate sampling events were conducted at Cane Break Pool between February 2002 and May 2003. Marron were marked by taking small triangular clips from their uropod margins. A pattern was followed from left to right on the ventral side of the uropods following a 1, 2, 4, and 7 combination for the single, double and triple figured numbers (Figure 3.2) This number pattern was derived from Abrahamsson (1965) who used it for cauterisation of the carapace of *Astacus astacus*. Treating males and females separately allowed for potentially greater than 1000 marron to be marked for each sex. Differentiating between marron types for males or females when marking individuals was not undertaken. These clips should persist over several moult cycles, allowing identification for an extended period after they have been initially marked.

On subsequent sampling events at Cane Break Pool, the numbering system was continued directly from the last male and female marron captured at the previous sampling event. If a captured marron had tail clips indicating it was a recapture, its number was recorded and its features measured and noted. As for all first time captured marron the location within Cane Break Pool that this marron was recaptured was recorded in order to measure its relative displacement.

Comparisons between recaptured *C. cainii* and *C. tenuimanus*

At the first sampling event at Cane Break Pool only the rostral carapace length (RCL) was recorded for marron captured and marked, that is, the orbital carapace length (OCL) was not recorded. For all other sampling events both the RCL and OCL were recorded. The OCL is known to be a more appropriate measurement for such types of study due to the damage marron can do to their rostra (Beatty *et al.* 2003). However, rather than convert the RCL measurements in Trip 1 to OCL using formulae such as that derived from Beatty *et al.* (2003), the RCL was used as the unit of size for all comparisons between *C. cainii* and *C. tenuimanus*. It was believed to be more appropriate to use a continuous set of real recorded values rather than include a subset of derived values. On a few occasions, marron would be captured that had damaged rostrums that prevented the measurement of RCL. Rather than remove these marron

from the data set, an RCL value was calculated from the OCL using a conversion factor of $RCL = 1.39 \times OCL$ taken from Morrissy (1970).

For each recaptured marron the set of parameters used to test the different replacement mechanism hypotheses was calculated based on a before and after approach. Growth rate, in mm/day, was estimated for each recaptured marron by dividing the difference in RCL between marking and recapture by the recapture interval. Displacement obtained by recaptured marron was estimated by measuring the distance, in metres (m), between the location of marking and recapture. Movement, in m/day, was estimated by dividing displacement by the recapture interval. The average size of each recaptured marron was calculated, based on the average of the RCL at the time of marking and time of recapture. A mean value was determined for each parameter for the particular type group of recaptured marron, then comparisons made.

To test hypotheses, a standard *t*-test was carried out to determine if the mean value for a particular parameter was significantly different at the 0.05 probability level between species (all individuals and between sexes) and also within species (between males and females).

Scatter plots were constructed to test for relationships between calculated growth rate and average RCL and between displacement and recapture interval of recaptured marron for *C. cainii* and *C. tenuimanus*. This was done to explore further the replacement mechanism hypotheses.

Size/frequency relationships

For all but two sampling events at Cane Break Pool, the RCL of all *C. cainii* and *C. tenuimanus* captured (including recaptures) was used to construct size/frequency histograms for both species. The RCLs of captured marron were grouped into 2.5mm arrays and the frequency of a particular array was measured as the percentage of marron within that array in relation to the overall number of *C. cainii* or *C. tenuimanus* captured. This was not done for hybrids due to there being too few caught to construct useful size/frequency histograms. An insufficient amount of marron caught on two trips did not allow practical size/frequency histograms to be constructed for *C. cainii* or *C. tenuimanus* for these sampling events.

An investigation of the replacement mechanism hypotheses related to growth and recruitment was undertaken from information obtainable from these histograms. Identification of individual cohorts, based on the modal peaks within histograms, was attempted in order to make comparisons between *C. tenuimanus* and *C. cainii*.

3.3 Results

3.3.1 Distribution Survey

The species profile for each site is presented in Table 3.1, with the basic water measurements taken and a general site description. The location of each site with respect to its position on the Margaret River, and a pie chart of the proportion of each marron type captured are presented in Figure 3.3. At sites where tissue samples were collected and marron genetically identified, any incorrect field identifications were corrected prior to determining species profiles. For all other sites, the species profiles are based solely on visual identification of marron carried out in the field. No adjustment was made to species profiles at sites based on the results of Chapter 2 regarding the level of accuracy in field identification. This was because it would not have changed the profiles significantly, nor would they have been necessarily genuine adjustments. For Cane Break Pool (site 17) the species profile is based only on the information obtained from marron the first time they were captured. Information from recaptured marron was not included in the species profile of Cane Break Pool.

The Margaret River is dominated by *C. cainii* throughout the middle and lower reaches (sites 1 to 12, see Table 3.1 & Figure 3.3). In these middle and lower reaches it was only at sites 1 to 3, all downstream of the townsite, where a very small proportion of the marron captured were *C. tenuimanus* or hybrids (Table 3.1, Figure 3.3). It appears that this region has the only downstream population of *C. tenuimanus*, which was not found to be present again until the onset of State forest in the upper reaches (site 13). There were no allopatric populations of *C. tenuimanus* discovered at any of the sites surveyed in this study. *C. tenuimanus* was found to occur only in sympatry with the introduced *C. cainii*.

The reappearance of *C. tenuimanus* was very abrupt, going from 0% of the marron captured at sites 4 to 12 to 46.3% at site 13 (Table 3.1) and represents a distinctive and abrupt boundary of occurrence for *C. tenuimanus* between the agricultural land use (site 12) and State forest (site 13). The proportion of *C. tenuimanus* captured at sites 13 to 20 was similar at around 40%, except for three notable sites. At site 16, three *C. tenuimanus* were captured out of a total of 55. This site consisted of three closely located pools, two of which were bordered on one side by farmland, and one pool that was surrounded by forest but was small in size with visibly turbid water. Site 15 was a relatively large sized pool, surrounded by state forest, which appeared in relative high-quality condition with very good riparian vegetation and clear deep water.

However, only 11.5 % of the 26 marron captured at this site were *C. tenuimanus*. There were no *C. tenuimanus* captured at site 21 (Table 3.1), which is the most upstream pool remaining during summer. There were, however, only ten marron caught from this site, which was very murky at the time that sampling took place (25th March 2002).

Clarity of pools appeared to be an extrinsic factor influencing the proportion of *C. tenuimanus* present and in the overall catch number in the upper reaches. There was little success in overall catch numbers of marron at site 21, two of the pools at site 16 and also at site 14, all of which contained turbid water. The water quality of these pools resembled that of some of the sites within the agricultural land use region of the middle reaches. Most notable was site 10, which was a long (about 400 m) deep pool that had very turbid water with degraded riparian vegetation and was bordered by farmland. Despite the apparent poor water quality and obvious degradation caused by the surrounding land use, some very large and apparently healthy *C. cainii* were caught there.

Extrinsic factors, such as degraded water, habitat and food quality, that might be associated with the pools of the middle reaches (which are surrounded by agricultural land use) may have influenced the more or less complete replacement of *C. tenuimanus* by *C. cainii*. This is in direct comparison to the better condition of most pools in the state forest of the upper reaches where substantial populations of *C. tenuimanus* occur (Figure 3.3). The question still remains as to what has been the degree of influence caused by the likely variation in extrinsic factors associated with the different sections of the Margaret River on the replacement of *C. tenuimanus* by *C. cainii*.

Table 3.1. Species profile of distribution sampling sites shown in Figure 3.3. Capture methods were baited traps (BT), drop/nets (DN) and scoop nets (SN). The total number (*n*) captured at each site and the proportion (%) and mean size (OCL \pm st. dev.) of male and female marron for each type is shown. Basic water measurements taken and a brief description of site condition at the time of sampling are given also.

Site	Date	Capture Method	n	C. cainii		Hybrid		C. tenuimanus		pH	Water measurements			Site Description. Foreshore grading (sites 1-12) from Margaret River Action Plan (CCCCG, 2003)	
				♂	♀	♂	♀	♂	♀		Con. (µs/cm)	DO (mg/l)	Temp. (°C)		
1.	11-12/12/02	BT, DN & SN	59	% OCL±SD(mm)	52.5 40.0 ±9.7	25.4 39.5 ±5.2	10.2 38.7 ±7.3	6.8 42.8 ±5.7	5.1 41.9 ±6.9	0 —	6.83	440	8.9	23.2	Fine and warm. Directly adjacent to residential properties and subject to water abstraction. Abundant fringing vegetation with clear water and rocky substrate in sections. B3 foreshore grading.
2.	30/10/02	BT	79	% OCL±SD(mm)	75.9 43.5 ±14.0	10.1 39.6 ±11.4	6.3 46.1 ±14.5	6.3 40.6 ±11.0	1.3 42.2 ±0.0	0 —	6.85	385	8.6	18.4	Overcast and drizzly. Some large fallen trees in parts. Riparian vegetation quite thin on northern bank. Water clear and habitat appeared abundant. C1 to C2 foreshore grading
3.	27/04/02	BT	93	% OCL±SD(mm)	47.3 44.7 ±13.0	49.5 42.3 ±14.2	0 —	1.1 41.0 ±0.0	0 —	2.2 31.2 ±0.8	—	—	—	—	Moderately overcast. Adjacent to residential properties on southern side with road separating houses. Very rocky substrate and quite shallow in places. Deep pool adjacent to 10m rocky waterfall. A2 to C2 foreshore grading.
4.	29-30/10/02	BT & SN	73	% OCL±SD(mm)	69.9 39.9 ±12.5	23.3 35.4 ±7.8	4.1 48.7 ±8.7	2.7 30.3 ±3.0	0 —	0 —	6.32	377	8.0	17.2	Overcast and drizzly. Large weir formally used as town's water supply. A3 foreshore grading.
5.	03/01/02	BT & DN	22	% RCL±SD(mm)	72.7 48.2 ±15.8*	27.3 43.5 ±16.3*	0 —	0 —	0 —	0 —	—	—	—	—	Fine and warm. Large woody debris in deep water. Numerous tracks along waters edge. Site on long section (2km) of permanent water. A3 to B1 foreshore grading.
6.	12-13/12/02	BT & DN	39	% OCL±SD(mm)	76.9 41.1 ±9.5	23.1 41.7 ±17.8	0 —	0 —	0 —	0 —	6.11	410	5.2	21.5	Fine and warm. Very good riparian vegetation. Isolated pool within national park. A2 to A3 foreshore grading.
7.	11/02/03	BT & SN	108	% OCL±SD(mm)	64.8 34.7 ±10.4	35.2 31.8 ±7.5	0 —	0 —	0 —	0 —	7.01	438	7.8	23.8	Cool and overcast with some drizzle. Large water storage dam (Ten Mile Brook Dam) operated by the Water Corporation. Town's water supply. Tourist destination.
8.	15-16/12/02	BT & DN	73	% OCL±SD(mm)	79.5 39.7 ±10.4	20.5 39.2 ±15.9	0 —	0 —	0 —	0 —	6.05	394	6.5	21.9	Fine and warm. River pool with vehicle access. National park on northern side. Several large rocks in water. Farmland on southern side but with good buffer zone of vegetation. A2 to B3 foreshore grading.
9.	14/12/02	BT	35	% OCL±SD(mm)	80.0 41.3 ±10.0	20.0 31.1 ±11.3	0 —	0 —	0 —	0 —	6.31	404	7.6	23.3	Fine and warm. Some large trees and fringing vegetation, but very narrow from waters edge. Large fallen tree in water. Water abstraction from pool. B1 to C2 foreshore grading
10.	26/04/02 & 05/05/03	BT	37	% OCL±SD(mm)	56.8 61.1 ±17.6	43.2 58.4 ±20.0	0 —	0 —	0 —	0 —	—	—	—	—	Cloudy and wet (26/4/02) and overcast and mild (7/5/03). Long pool with farmland adjacent to both sides. Water very turbid for both sampling events. B3 to C2 foreshore grading.
11.	15/12/02	BT	21	% OCL±SD(mm)	57.1 52.8 ±19.4	38.1 54.2 ±13.8	4.8 62.5 ±0.0	0 —	0 —	0 —	6.02	367	7.2	21.0	Fine and warm. Water abstraction from river pool. Some large trees present at waters edge. Very rocky substrate at upstream margin of pool. B2 to C1 foreshore grading
12.	06/05/02	BT	20	% OCL±SD(mm)	50.0 56.1 ±19.5	45.0 64.4 ±17.5	5.0 67.8 ±0.0	0 —	0 —	0 —	6.31	455	6.6	17.3	Overcast and cool. Small sized pool on boundary of farmland and State Forest. Riparian vegetation in good condition. Limited to small buffer zone on farmland side. Water quite clear.
13.	16-17/12/02	BT & DN	162	% OCL±SD(mm)	34.6 39.1 ±9.2	17.3 38.8 ±6.5	1.2 46.0 ±3.5	0.6 49.7 ±0.0	30.9 35.5 ±6.6	15.4 33.0 ±6.0	6.07	379	8.5	23.5	Fine and warm. First pool (moderate size) in State Forest. Good natural condition, although easy vehicle access and evidence of human disturbance (eg. campsite). Water very clear and fresh.
14.	01/04/02	BT	30	% OCL±SD(mm)	20.0 49.1 ±9.1	43.3 47.1 ±15.7	3.3 78.0 ±0.0	3.3 47.3 ±0.0	23.3 43.9 ±7.5	6.7 41.6 ±2.1	6.20	405	6.2	19.6	Overcast and mild. Small sized pool with water fairly murky. Large tree trunk half submerged. Rocky banks in some places. Vehicle access with evidence of human disturbance and camping.
15.	31/03-01/04/02	BT & DN	26	% OCL±SD(mm)	61.5 34.9 ±12.0	19.2 35.0 ±15.0	0 —	7.7 52.3 ±12.9	7.7 46.0 ±8.5	3.8 35.9 ±0.0	6.28	435	8.4	20.6	Overcast and mild. Large pool with clear water and very good riparian vegetation. Submerged large rocks (boulders) and tree trunk. Vehicle access with evidence of human disturbance and camping. Illegal marron trap found.
16.	30-31/4/02, 26/04/02 & 07/05/03	BT & DN	55	% OCL±SD(mm)	43.6 60.8 ±23.1	38.2 48.9 ±14.9	1.8 No data	10.9 No data	5.5 57.9 ±9.4	0 —	6.12 – 6.22	364 – 435	5.5 – 6.1	19.3 – 20.7	Series of 3 pools (2 small 1 moderate size). Farmland encroaches riparian zone of two most upstream pools. All in fairly good condition, but 2 pools murky. Evidence of prior livestock disturbance. Data for hybrid marron was lost.
17.	02/02-05/03	BT, DN & SN	479	% OCL±SD(mm)	27.1 46.3 ±9.4	23.4 46.2 ±12.4	5.0 47.0 ±10.7	3.8 52.6 ±9.4	24.2 43.6 ±8.3	16.5 43.2 ±8.0	—	—	—	—	Cane Break Pool. Tourist destination with multiple campsites and picnic areas. Used for swimming also. Managed by DCLM and still in good condition with very clear water.
18.	17/05, 17/06 & 29/10/02	BT, DN & SN	95	% OCL±SD(mm)	23.2 44.5 ±10.3	23.2 45.5 ±10.8	4.2 41.1 ±6.8	4.2 49.1 ±13.0	24.2 40.5 ±9.2	21.1 45.1 ±8.6	5.32 – 5.86	320 – 350	8.1	11.4 – 15.8	Moderate size pool in good natural condition. Water very clear with rocky sections and submerged tree trunks. Easy access with campsite adjacent. Human disturbance evident.
19.	28-29/04 & 29/10/02	BT & DN	75	% OCL±SD(mm)	21.3 43.6 ±9.1	26.7 41.8 ±9.6	2.7 36.5 ±5.5	5.3 36.9 ±5.3	32.0 43.7 ±10.3	12.0 37.4 ±7.0	5.58	287	7.4	15.9	Fine (29/03/02) and overcast and drizzly (29/10/02). Most downstream section of long pool. Good natural condition. Campsite and human disturbance evident
20.	30/04/02	BT	61	% OCL±SD(mm)	24.6 48.8 ±8.1	24.6 48.8 ±7.6	14.8 51.1 ±9.5	6.6 44.8 ±8.1	14.8 47.7 ±7.1	14.8 46.9 ±6.3	5.75	473	6.8	20.1	Mostly fine and mild. Long pool in very good condition. Only moderate human disturbance evident in some parts. Water very clear and fresh. Deep in sections
21.	25/03/02	BT	10	% OCL±SD(mm)	30.0 47.5 ±9.2	60.0 49.5 ±7.9	10.0 66.9 ±0.0	0 —	0 —	0 —	6.06	468	4.9	15.0	Cloudy and wet. Small size pool adjacent to gravel road and campsite. Human disturbance evident. Water very murky. Most upstream pool remaining during summer.

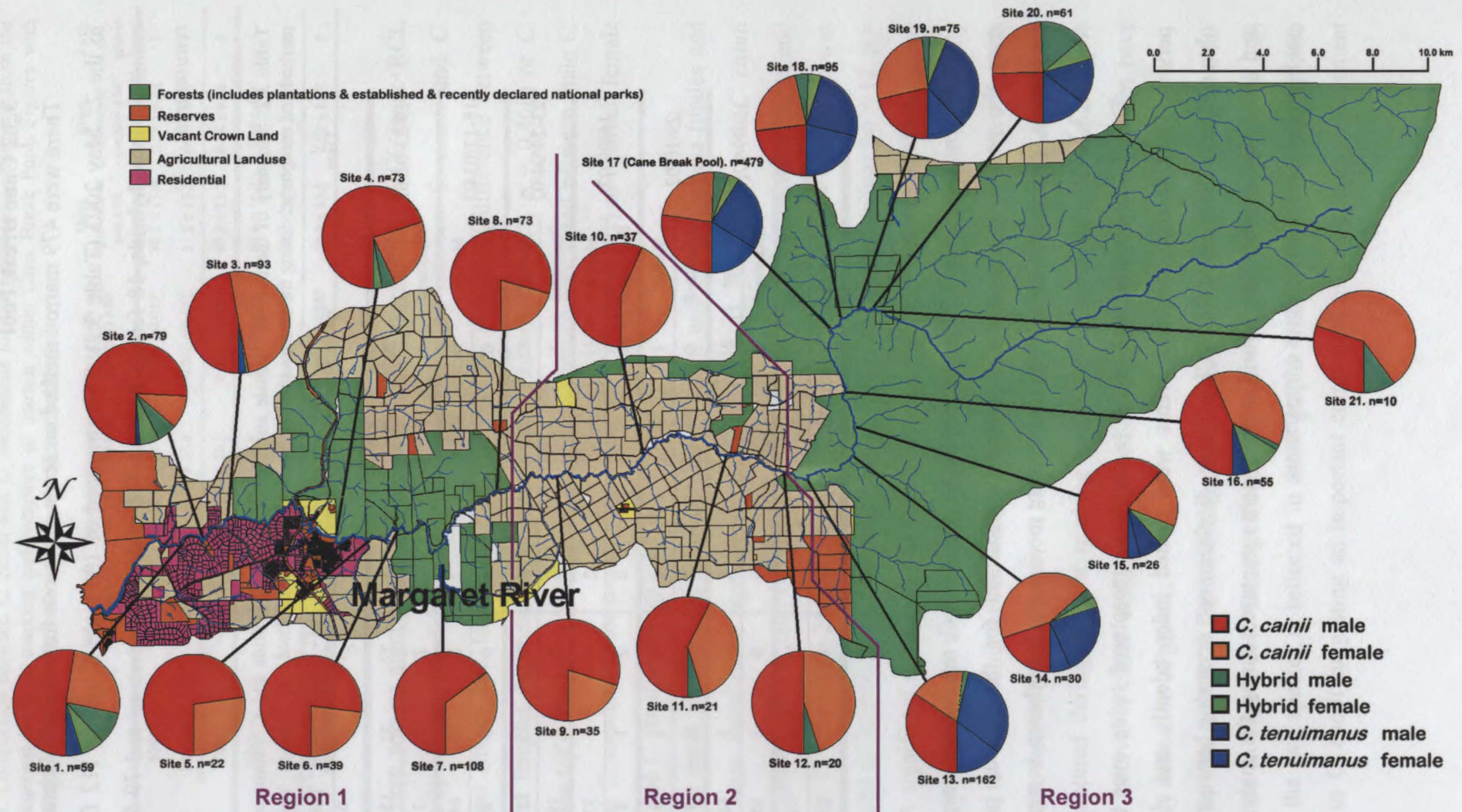


Figure 3.3. Distribution of marron species within the Margaret River, with catchment area and associated land use. Pie charts represent the proportion of male and female *C. cainii*, hybrid and *C. tenuimanus* marron captured at each distribution sampling site. The site number and overall amount of marron captured is shown beneath each pie chart. The boundaries of the three distinct conservation regions are shown (see Discussion). Source of background map: Adapted from Delaney and Gardner (Delaney and Gardner 2000).

3.3.2 Cane Break Pool

There were 479 marron marked on seven occasions from the 19th February 2002 to the 7th May 2003 (Table 3.2). This consisted of 130 male *C. cainii*, 112 female *C. cainii*, 24 male hybrids, 18 female hybrids, 116 male *C. tenuimanus* and 79 female *C. tenuimanus*.

Table 3.2. Summary of the number of male and female *C. cainii*, hybrids and *C. tenuimanus* marron marked and recaptured during the seven sampling events at Cane Break Pool.

Date		<i>C. cainii</i>		Hybrid		<i>C. tenuimanus</i>		Total n
		♂	♀	♂	♀	♂	♀	
19-23/02/02	Marked	29	36	6	4	27	22	124
	Recaptured	0	0	0	0	2	0	2
15-17/03/02	Marked	18	17	8	7	8	14	66
	Recaptured	0	0	0	0	0	0	0
23-24/04/02	Marked	42	26	5	4	35	25	137
	Recaptured	3	2	1	0	2	2	10
16/08/02	Marked	11	9	0	2	7	4	33
	Recaptured	2	0	0	0	2	1	5
29/10/02	Marked	1	1	0	0	5	1	8
	Recaptured	0	0	0	0	0	0	0
18-19/12/02	Marked	21	16	1	2	17	3	60
	Recaptured	6	6	0	0	5	1	18
06-07/05/03	Marked	12	8	2	4	15	10	51
	Recaptured	4	0	0	1	3	2	10

Recaptured marron

There were 23 and 20 recaptures for *C. cainii* and *C. tenuimanus*, respectively, over the period of the study. A species comparison for the various population parameters, used to test some of the replacement mechanism hypotheses, is summarized in Table 3.3.

One *C. tenuimanus* and one *C. cainii* were recaptured twice over the study period. Their second recapture events were treated independently and data were obtained from differences (i.e. RCL increase, displacement) occurring between the first and second recaptures. Two hybrid marron were recaptured during the study but have not been included in any species comparisons.

Table 3.3. Comparison between *C. cainii* and *C. tenuimanus* for the parameters calculated from the changes observed between marking and recapture of marron within Cane Break Pool. Figures with corresponding symbols were those found to be significantly different.

		<i>n</i>	Mean 'average' RCL (mm) \pm SE	Mean mark-recapture Interval (days) \pm SE	Mean growth rate (mm/day) \pm SE	Mean displacement (m) \pm SE	Mean movement (m/day) \pm SE	# with zero growth
<i>C. cainii</i>	Male	15	67.3 \pm 2.8	204.6 \pm 30.1	0.064 \pm 0.013 [^]	86.0 \pm 17.6	0.75 \pm 0.24	2
	Female	8	72.3 \pm 6.0	211.4 \pm 38.9	0.060 \pm 0.012	31.9 \pm 11.5	0.26 \pm 0.11	1
	Total	23	69.0 \pm 2.7	207.0 \pm 23.3	0.063 \pm 0.009 [*]	67.2 \pm 13.2	0.58 \pm 0.16	3
<i>C. tenuimanus</i>	Male	14	66.7 \pm 3.5	211.9 \pm 40.1	0.028 \pm 0.01 [^]	83.6 \pm 16.0	3.29 \pm 1.9	7
	Female	6	62.0 \pm 2.1	221.8 \pm 58.8	0.046 \pm 0.016	68.3 \pm 26.9	0.72 \pm 0.46	0
	Total	20	65.3 \pm 2.5	214.9 \pm 32.3	0.033 \pm 0.008 [*]	79.0 \pm 13.5	2.52 \pm 1.35	7

There was no significance difference for mean average size (RCL mark+RCL recapture/2) of recaptured marron ($df = 41$, $p = 0.325$) between *C. cainii* and *C. tenuimanus* (males and females). There was also no significant difference between males and females for mean average size for *C. cainii* ($df = 21$, $p = 0.468$) or *C. tenuimanus* ($df = 18$, $p = 0.264$). No significant difference was found between male *C. cainii* and *C. tenuimanus* for mean average size ($df = 27$, $p = 0.901$) or between female *C. cainii* and *C. tenuimanus* ($df = 12$, $p = 0.279$).

The mean growth rates (mm/day) for *C. cainii* and *C. tenuimanus* (males and females) were found to be significantly different ($df = 41$, $p = 0.023$) with *C. cainii* having a greater growth rate. No significant difference was found between males and females for *C. cainii* ($df = 21$, $p = 0.816$) or *C. tenuimanus* ($df = 18$, $p = 0.373$), nor was a significant difference found between female *C. cainii* and *C. tenuimanus* ($df = 12$, $p = 0.494$). A significant difference was found between male *C. cainii* and *C. tenuimanus* ($df = 27$, $p = 0.034$) for mean growth rates. This significant difference is most likely attributed to there being seven male *C. tenuimanus* recaptures with zero growth compared to only two for *C. cainii*. It should be noted that two marked *C. tenuimanus* were recaptured during the first marking event. With only a three day marking to recapture interval it could easily be expected that these two marron would not have shown any indication of growth.

The change in RCL from marking and recapture for *C. cainii* and *C. tenuimanus* is presented in Figure 3.4. The significant difference in growth rate (see Table 3.3) between the two species is reflected in these graphs with the lower number of zero growths in *C. cainii* suggesting an important component of this species replacement ability.

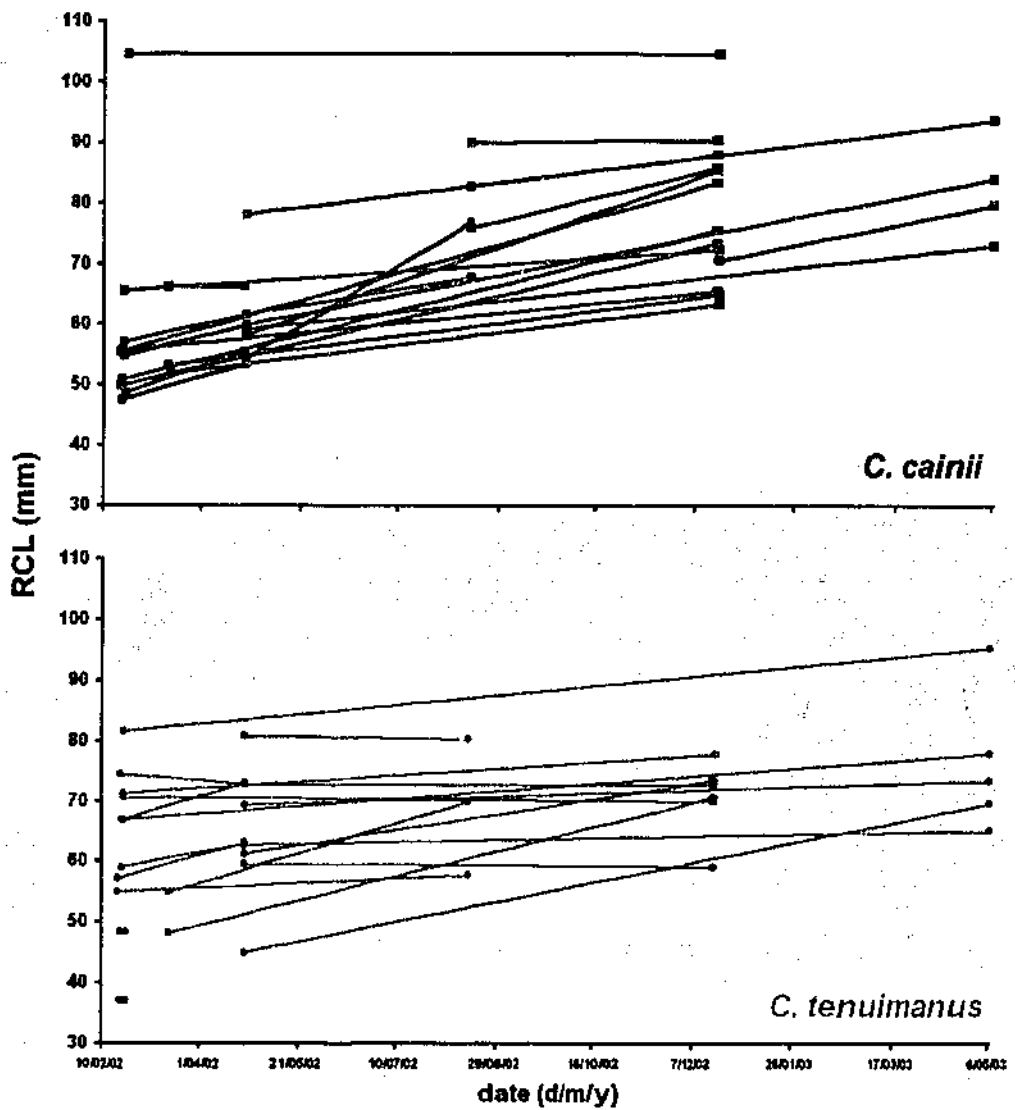


Figure 3.4. Change in rostral carapace length (RCL) from date of original capture and marking to date of recapture for *C. cainii* and *C. tenuimanus*.

There was no significant relationship between growth rate and average RCL of recaptured marron for *C. cainii* ($r^2 = 0.172$) or *C. tenuimanus* ($r^2 = 0.004$) (Figure 3.5).

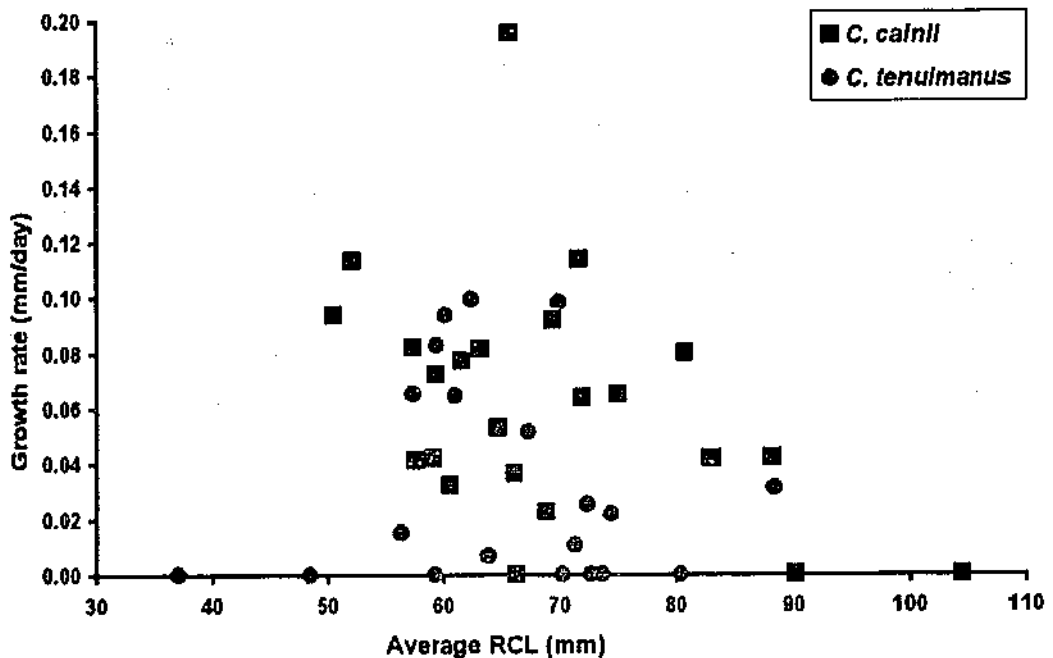


Figure 3.5. Relationship between growth rate (mm/day) and average RCL (mm) of marked and recaptured *C. cainii* and *C. tenuimanus* in Cane Break Pool. Average RCL is calculated as the average between RCL at marking and RCL at recapture.

No significant relationship was found between displacement and recapture interval (Figure 3.6) for *C. cainii* ($r^2 = 0.013$) or *C. tenuimanus* ($r^2 = 0.001$). As the actual movement patterns of marron were not measured it is difficult to make comparisons from this (lack of) relationship. Marron of both species could have moved much greater distances during their marking to recapture interval than what is represented in their displacement. The two *C. tenuimanus* that were caught only three days after marking had both moved about 60 m, which gave them a movement rate of 20 m/day. This was very much greater than calculated for any other marron and created a distorted mean value for *C. tenuimanus* (Table 3.3).

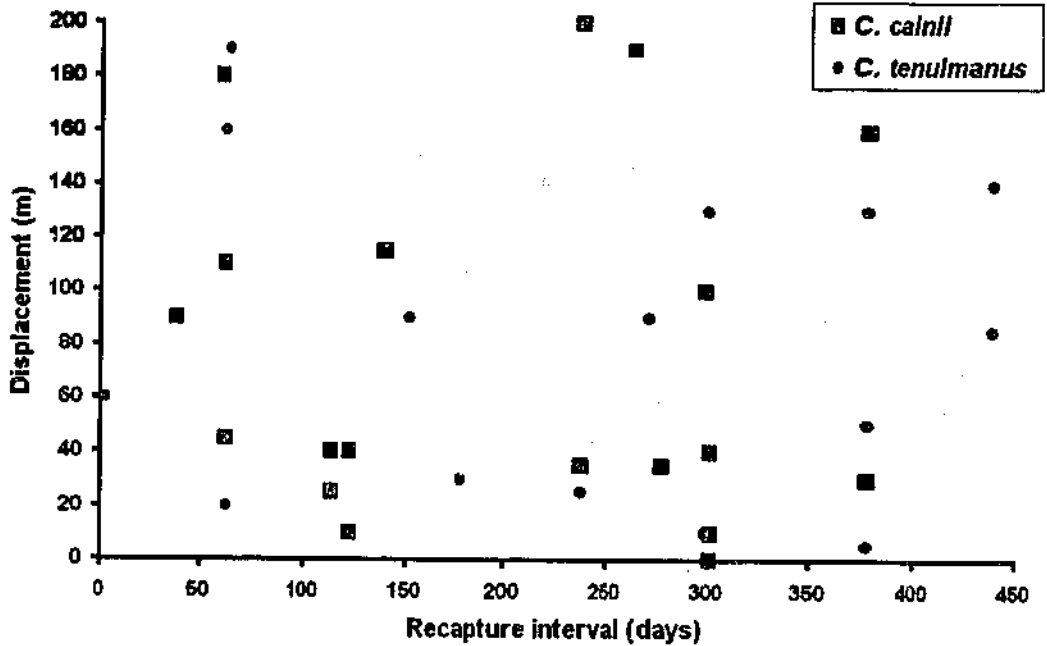


Figure 3.6. Relationship between the displacement (m) and interval (days) from marking and recapture of *C. calnii* and *C. tenuimanus* in Cane Break Pool.

Size/frequency comparisons

A comparison of size (RCL)/frequency (%) relationships over time between *C. calnii* and *C. tenuimanus* captured at Cane Break Pool, during the mark-recapture study, are presented in Figure 3.7. Trip 4 (16/8/02) and Trip 5 (29/10/02) were omitted as only 39 and 8 marron overall were captured respectively, resulting in an insufficient number of marron to construct adequate size frequency histograms.

In a single sample size/frequency histogram, growth can be estimated from the distance between modal peaks and the time intervals they represent, i.e. they correspond to cohorts (King 1995). Large sample sizes make it easier to distinguish cohorts, as these will tend to group together to form modal peaks. However, when different cohorts bunch together in size/frequency histograms so that modes are not evident, it is not possible to estimate growth rate from these data (King 1995). This is what can happen when growth rates decrease as an animal ages, such as in marron. This factor combined with recreational fishing, which removes the largest marron each year, can make it difficult to distinguish cohorts for the older and larger sized marron in populations. The larger and older marron in different cohorts will group in size/frequency histograms as a result of slower growth rates and reduced population numbers.

There is no single size/frequency histogram in Figure 3.

7, in which there is more than one clear modal peak representing suspected cohorts, for either species, to allow an estimate of growth rate to be made. There are,

however, some single modal peaks within the size/frequency histograms for both species that may represent cohorts. It is possible to track a single cohort over progressive size/frequency histograms and produce an estimate of growth rate from the changing size values of the cohort over the time intervals (King 1995). However, the problem is that corresponding cohorts are not clearly defined over progressive sampling events in size/frequency histograms for both species. This lack of definition makes it difficult to produce a plot of size increase over time, which is required to produce the estimate of growth rate (King 1995).

For *C. cainii* a growth rate may be loosely estimated by comparing Trip 1 (19/02/02) with Trip 3 (24/04/02). Two modal peaks are present in Trip 1 at the 50 mm and 52.5 mm RCL arrays, which average out to 51.25 mm. Trip 3 has a possible corresponding modal peak at the 60 mm RCL array suggesting a 8.75 mm increase in RCL for this potential cohort. A time interval of 64 days between Trip 1 and Trip 3 equates to an estimated growth rate of 0.14 mm/day. In the case of *C. tenuimanus* there is an average modal peak value in Trip 2 (16/03/02) of 48.75 mm RCL, which may represent a cohort. The corresponding cohort is possibly evident in Trip 6 (18/12/02) with a modal peak value of 60 mm RCL, which suggest an increase of 11.25 mm. Taking the 302-day time interval into account produces an estimated growth rate of 0.04 mm/day, very much less than that calculated for *C. cainii*. However, any attempt to compare directly these estimated growth rates for *C. cainii* and *C. tenuimanus* from the progressive size/frequency histograms in Figure 3.7 will be unreliable. The small sample sizes for separate trips have made it very difficult to fittingly define corresponding cohorts, within and between species, so that comparisons can be made. Although some successive modal peaks that may represent the same cohort appear to be evident for both species, they are not in the same trips and have different time intervals. As crayfish have intermittent growth, corresponding time intervals would be needed. This would be the only way to achieve a reliable comparison between *C. cainii* and *C. tenuimanus* for growth rate in matching cohorts from a size/frequency histogram.

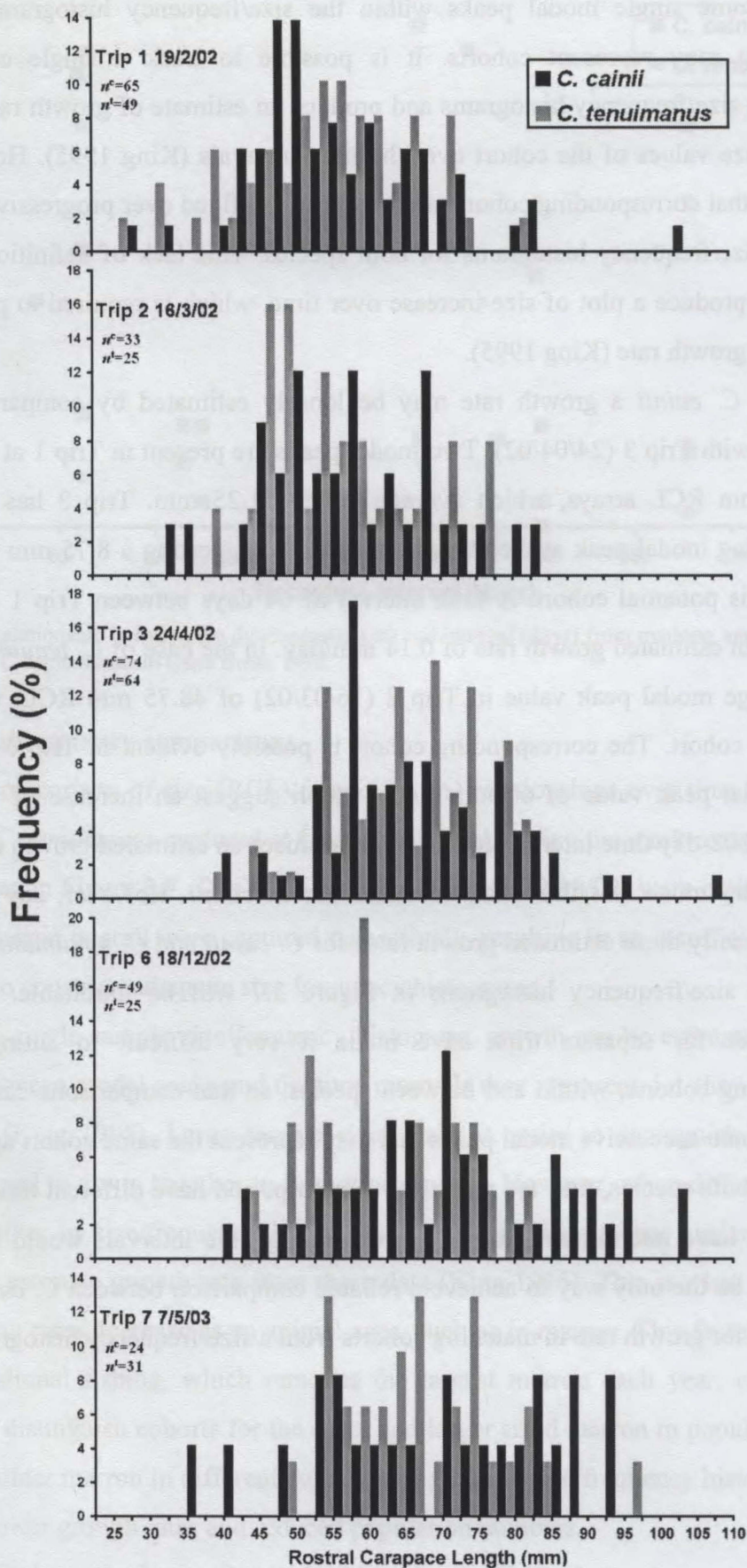


Figure 3.7. Size frequency histograms for *C. cainii* and *C. tenuimanus* captured at Cane Break Pool during the mark-recapture study. Only 5 of 7 sampling events are included as insufficient numbers were caught at the other two. Both species are included in each histogram with the sample sizes for *C. cainii* (n^c) and *C. tenuimanus* (n^t) and the starting date of each sampling event presented.

Another issue related to testing the replacement mechanism hypotheses, is the earlier recruitment of the *C. cainii* into a catchable size range. The *C. cainii* captured on trip 1 had a 51.25 mm RCL average modal peak value, compared to *C. tenuimanus*, which has a value of 48.75 mm RCL in Trip 2, 25 days later. Although these sizes do not represent YOY crayfish, it may loosely support the hypotheses related to greater growth and earlier brood release in *C. cainii*.

More, larger sized *C. cainii* were captured during Trip 6 in mid December 2002 (Figure 3.7) and more were captured overall (49 *C. cainii* compared to 25 *C. tenuimanus*). Only four female *C. tenuimanus* were captured including one (RCL=70.6 mm) that was berried with attached eggs. However, 22 female *C. cainii* were captured and included two (RCL=104.7 mm & 72.3 mm respectively) that were carrying hatched juveniles. The lack of *C. tenuimanus* females could suggest that most were in an inactive state due to being berried, which would reduce their chance of capture (Morrissy 1970). The almost equal ratio of males to females for *C. cainii* might suggest that juveniles had already been released from most mothers. Beatty *et al.* (2003) found the release of most *C. cainii* juveniles to peak between mid-November and mid December in Lake Naverino (Waroona Dam), located about 100 km south of Perth. This period was found to be earlier than with specimens from the more southern Warren River (about 150 km East of Margaret River) (Morrissy 1975b) and was attributed to an earlier rise and overall higher water temperature (Beatty *et al.* 2003). Being an isolated pool during the summer months with no flow and little shade, Cane Break Pool could be expected to also have an earlier rise and overall higher water temperature than the Warren River. This expectation may validate the possibility that the majority of *C. cainii* juveniles had been released from their mothers in Cane Break Pool at the time of Trip 6 in mid-December. Therefore, female *C. cainii* could be expected to have a relatively equal chance of capture as males (Morrissy 1970). Given the assumption that female *C. tenuimanus* were at an earlier stage of spawning, which resulted in their low catch rate compared to males, it is plausible that the release of juveniles would then be later than for *C. cainii*.

Trip 7 (07/05/02) may represent the possible effect that the 2003 ban on recreational fishing upstream of the Ten Mile Brook junction had on the occurrence of large marron. Combining all types of marron, including hybrids (not represented in Figure 3.7), there was a greater frequency of marron above the legal 76 mm RCL caught in Trip 7 (33.5 %, $n = 22$) compared to Trip 3 (21.6 %, $n = 32$). Trip 3 was undertaken just over two months following the closure of the 2002 recreational marron fishing

season. Trip 7 was undertaken almost a year later, with the difference being the 2003 ban on recreational marron fishing in the upper reaches of the Margaret River had been in place. A one-tail t test revealed that the mean RCL for all marron captured in Trip 7 ($70.0 \text{ mm} \pm 1.75$) was significantly greater than in Trip 3 ($66.5 \text{ mm} \pm 0.98$) ($df = 209$, $p = 0.032$).

Separately, *C. cainii* and *C. tenuimanus* showed some differences. For Trip 3, 24.3 % ($n = 18$) of *C. cainii* captured were above the legal 76 mm RCL compared to 37.5 % ($n = 9$) for Trip 7. For *C. tenuimanus*, 14.1 % ($n = 9$) of those captured were above the legal 76 mm RCL during Trip 3 compared to 19.4 % ($n = 6$) during Trip 7. Statistically, there was no significant increase in mean RCL between Trip 3 and Trip 7 for *C. cainii* ($df = 97$, $p = 0.234$) or *C. tenuimanus* ($df = 94$, $p = 0.162$), as revealed by one-tail t tests. Of note is that during Trip 3 only 50 % ($n = 5$) of the hybrid marron captured (hybrids were not included in Figure 3.5) were above the legal 76 mm RCL compared to 100 % ($n = 7$) during Trip 7. This is the likely reason why there was a significant increase in mean RCL from Trip 3 to Trip 7 for all marron captured. There is a strong likelihood that recreational fishing plays a role in removing hybrids from populations.

3.4 Discussion

3.4.1 Distribution of Marron Species within the Margaret River

Key Findings

1. *C. tenuimanus* is almost completely restricted to state forest of the upper reaches with a very small population downstream of the Margaret River townsite.
2. *C. tenuimanus* is found only in sympatry with the introduced *C. cainii*.
3. There is an abrupt reappearance of *C. tenuimanus* with the onset of its distribution in the forested upper reaches.
4. Differences between sections of the Margaret River, associated with extrinsic factors, appear to influence the replacement ability of *C. cainii* and the distribution and abundance of *C. tenuimanus*.

Regional Division of Margaret River

The Margaret River can be regionally divided to give lower, middle and upper reaches. The boundaries of these regions are designated based on possible differences in extrinsic factors related to variation in land use, and results of the distribution survey. The three regions are likely to have specific issues in regards to the conservation of *C. tenuimanus*.

Region 1. Lower Reaches.

The boundary of this region (Figure 3.3) is based on the upstream border of the proposed Bramley National Park and the onset of agricultural land use. The majority of the river foreshore in this region is within some form of protected area such as council reserve or national park. However, residential areas border about half of the region and there are numerous tracks and access points that run almost the entire length of the lower reaches.

The Cape to Cape Catchments Group, in their action plan for the Margaret River, which used the Foreshore Condition Assessment method developed by Pen and Scott (1995), rate the condition of almost half of the foreshore in region 1 as A grade (CCCG 2003). The remainder of region 1 is rated as B grade; however, this is mostly low level weed infestation. Only a small proportion was rated as C grade, in that it is erosion prone. Even though many of the pools in region 1 appear comparable in quality to those in the upper reaches (pers. obs.), the same is not reflected in the occurrence of *C. tenuimanus*. A possible explanation for this could be an assumed greater length of

time that *C. cainii* has been present in the lower reaches compared to the upper reaches (Austin and Ryan 2002). *C. cainii* may simply have had the necessary time to replace almost completely *C. tenuimanus* through differences in intrinsic factors between the species, without the influence of a degraded environment. The pools located in Region 1 all appear to have suitable habitat for marron. Two of the three pools samples at Site 3 are relatively shallow and the riverbed consists mainly of medium to large sized rocks that would form abundant shelter for marron. This type of habitat is present throughout Region 1, although it is currently occupied almost exclusively by *C. cainii*.

Only three sites (1 to 3) surveyed within Region 1 were found to contain *C. tenuimanus*, all in low proportions. It is unclear if these marron are the offspring of parents still inhabiting these sites or they have arrived there as juveniles from the upper reaches. However, a relatively high proportion of hybrids were captured at these sites (see Table 3.1), which suggests likely local matings between *C. cainii* and *C. tenuimanus*. Given that *C. tenuimanus* was not captured again until the start of Region 3 (Figure 3.3, see site 13), it is most likely that those marron found in the lower reaches are part of a very small remnant population. Had they moved down from the upper reaches as juveniles, it could be expected that some *C. tenuimanus* would have also been caught between site 3 and site 13.

If the limited number *C. tenuimanus* in these lower reaches are part of a remnant population, they may represent some level of genetic divergence. Imgrund (1998), as part of a broader population genetic study of marron, sampled marron specimens from the lower and upper reaches of the Margaret River for genetic analysis, using microsatellite loci. At the time of this study the hairy marron was not recognised as a separate species and all marron were known taxonomically as *C. tenuimanus*. The hairy marron was referred to as a morphotype alongside another within the Margaret River. This other morphotype is now recognised as *C. cainii* and was translocated into the Margaret River (Austin and Ryan 2002). Imgrund *et al.* (1997) claimed that hairy marron in the lower reaches were diverging from the upstream population of hairy marron and were becoming increasingly genetically similar to the introduced marron in the lower reaches. It is now argued that *C. tenuimanus* is being replaced and not genetically assimilated in the lower reaches (Austin and Ryan 2002). Regardless of this, the remaining *C. tenuimanus* within the lower reaches may represent genetic variation from those in upper reaches (Imgrund 1998), and in the absence of genetic exchange between the lower and upper reaches, this remnant population requires protection in its own right.

It could be recommended that any *C. tenuimanus* captured from the lower reaches should be retained for the purposes of establishing a captive population. Any juveniles produced from this captive population could be used for reintroduction into the lower reaches, following control measures such as active removal of *C. cainii*. Reintroduction of *C. tenuimanus* into these sites should come from a captive population originating from the same location. This will help in maintaining as much genetic variation as possible within *C. tenuimanus*. It could be difficult, however, to find appropriate numbers for a captive population without severely impacting on the existing small population. However, the protection of genetic variation within this threatened species may be more important.

Before any plans to protect the apparent remnant population of *C. tenuimanus* in the lower reaches as a separate entity, their actual genetic separation from the upper reaches should be verified. The first step is to establish if they are genetically distinct from the upper reaches, and secondly, the possibility that the remnant population of *C. tenuimanus* in the lower reaches is the result of translocation from the upper reaches needs to be established. There is the chance that persons fishing in the upper reaches translocated marron, including *C. tenuimanus*, in an attempt to boost population numbers in the lower reaches.

There are weirs present in Region 1 that have created areas capable of supporting substantial populations of marron. Site 6, in particular, resembles a large water storage dam and was the town's main water supply. A newer impoundment, Ten Mile Brook dam (site 7), will have an important role to play in the conservation of *C. tenuimanus*. Ten Mile Brook dam is a sizeable water body and capable of maintaining a very large population of marron. At present this population consists entirely of *C. cainii* and probably has done so since construction of the dam in 1995. There is a big issue in the risk that this dam could play in continued introduction of *C. cainii* into the Margaret River. Overflow from the dam during peak flows could easily allow *C. cainii* to enter the Ten Mile Brook, which leads directly to the Margaret River. It may be possible to construct barriers that prevent marron from escaping the dam without restricting water flow. However, stopping juvenile marron from escaping would be difficult as a barrier such as a small size mesh grid would be likely to hinder water flow.

Region 2. Middle Reaches.

This section (Figure 3.3) is the most degraded (CCCG 2003) with agricultural land use bordering both sides of the river. It appears that *C. cainii* has totally replaced

C. tenuimanus from the middle reaches of the Margaret River. No *C. tenuimanus* were captured at any of the four sites (9-12) within Region 2.

Site 10 was at the most upstream third of a wide, deep pool about 600 m long. Most of the foreshore was given a C grade rating by the Cape to Cape Catchments Group with a small proportion receiving a low B grade (CCCG 2003). There was no flow at the time of sampling and the water appeared very turbid. However, this site produced some of the largest *C. cainii* captured throughout the entire study, including the biggest, a female at 94.0 mm OCL. Several other *C. cainii* of comparable size were also captured at this site, although the overall catch number was low. The baited traps were spread out over about 200 m, so it is hard to estimate density but this site does appear to have a significant population of quite large *C. cainii*.

Only two hybrids were captured throughout this region, one at site 11 and the other at site 12. The catch result at site 12 (see Table 3.1 and Figure 3.3) was surprising as it was anticipated that *C. tenuimanus* would be captured. This was due to the close proximity of site 13 in Region 3, where almost half the marron captured were *C. tenuimanus*. Site 12 appeared to be in relative good condition with clear water and state forest bordering about half of this pool. However, only twenty marron were captured at this site with traps spread evenly around the pool's perimeter. The apparent low density of marron present is hard to explain, as the nearby farmland does not appear to affect the condition of this pool. This pool has substantial riparian vegetation, although there is clear human disturbance in the form of a small landing and part of a bank is reinforced with car tyres. This site also had very easy access from different unsealed roads. It is likely that the influence of the adjacent land use on the extrinsic properties of the pool could be reflected in the overall low number of marron present and a lack of *C. tenuimanus*.

Region 3. Upper Reaches.

This region is contained within state forest and almost all the pools surveyed could be described as being in A grade condition in relation to the grading given by the CCCG for the lower and middle reaches (CCCG 2003). The forested upper reaches were not included in the action plan for the Margaret River (CCCG 2003) and the boundary between Region 2 and 3 in this present study coincides directly with the upstream limit of foreshore assessment. Although the catchment extends much further than the most upstream site surveyed (site 21) there are no permanent pools upstream from that location. This region was the only one found to contain substantial

populations of *C. tenuimanus*, although only in sympatry with *C. cainii*. At present *C. tenuimanus* appears to coexist with *C. cainii* in this region, but the likelihood of this situation continuing is unclear.

The first site in this region (13) was a contrast to those within the middle reaches, both in quality and presence of *C. tenuimanus*. Despite this site being only a short distance (about 900 m) upstream from site 12, with no other pools in between, the reappearance of *C. tenuimanus* was dramatic; the proportion captured changed from 0 to 46.3 %. A likely explanation at this time appears to be the abrupt change to forest and better river condition.

Site 15 was unusual for Region 3 with only a small proportion of *C. tenuimanus* captured. Site 15 was easily accessible with a 4WD vehicle and had a small campsite with bush tracks around the edge of the pool. This site was obviously used frequently during the recreational marron fishing season and presumably for illegal fishing. Numerous bait lines were evident from positions on the edge of the riverbank and a large steel marron trap (illegal) was found hidden nearby in bush. Despite being a large sized pool in essentially A grade condition with clear deep water and bordered by state forest, successful catch rate was not achieved. The low overall catch number ($n = 26$) and small proportion of *C. tenuimanus* (11.5 %) may be a reflection of excessive marron fishing. This would be in relation to a possible greater capacity that *C. cainii* has for population recovery and growth, as suggested by the mark-recapture study at Cane Break Pool (see Section 3.4.3).

Given the location of site 16 (Figure 3.3) it would appear that even a small influence from agricultural land use on the extrinsic condition of pools could affect the ability of *C. tenuimanus* to coexist with *C. cainii*. Site 16, like site 15, was also unusual for Region 3 with only three (5.5 %) out of 55 marron captured being *C. tenuimanus*. Farmland bordered the two most upstream pools with the third pool only about a further 150 m downstream within state forest. The adjacent farmland was completely fenced with the paddocks being used for only a few head of cattle and horses and also for a light aircraft runway. There was relative easy access to site 16 with a gravel road on the farmland side leading downhill straight to the central pool, which had evidence of prior livestock disturbance. The most upstream pool at site 16 has somewhat more difficult access. A 4WD vehicle is required for direct access to this pool, although not from the aforementioned gravel road. The small size of these pools could have been a further extrinsic property that worked in association with the influence of agricultural land use to increase the replacement of *C. tenuimanus*. In a small sized pool, food and shelter

will likely become more limited resources, increasing the competition for them. Extrinsic factors related to the size and condition of these pools may allow *C. cainii* to dominate over that of the general trend in the forests of the upper reaches, where *C. tenuimanus* makes up a significant proportion of the marron present.

One noteworthy observation during the surveying of sites in Region 3 was the occasional disproportionate ratio of *C. cainii* or *C. tenuimanus* within individual baited traps. At sites where both species were well represented in the overall marron population, some traps that were hauled contained almost entirely one species or the other (pers. obs.). Although this was not a common occurrence, it does suggest the possibility of some sort of differing habitat association and segregation for the different species, within the same river pool. This factor could be an important issue in the replacement ability of *C. cainii*, and should receive greater attention in any future research in this region.

Most of the pools in region 3 offer good opportunity for illegal marron fishing due to their seclusion and relative ease of access with off-road vehicles; some are even accessible with standard cars. Discussions with local landowners, who said to have witnessed suspected illegal marron fishing taking place, support this statement. Site 17 (Cane Break Pool) is an official campsite operated and maintained by the Department of Conservation and Land Management (DCLM) and is well known to locals and tourists. During several sampling events at this site, there were no other persons staying at the camping grounds. However, at other times, particularly holiday periods, the campsite was full of campers. Despite the greater opportunity for legal (now banned from this pool) and illegal marron fishing, the relative proportions of *C. cainii* and *C. tenuimanus* do not appear to be much different from other comparable sites in Region 3.

Summary of Distribution

The forested upper reaches (Region 3) was the only section of Margaret River found to have substantial populations of *C. tenuimanus*, although this species was only found in sympatry with *C. cainii*.

The upstream boundary of Region 2 with Region 3 represents an intriguing location within the Margaret River. The abrupt reappearance *C. tenuimanus* at the onset of state forest in Region 3 (Figure 3.3, see site 13) brings about questions in regards to the extrinsic properties within Region 2, and Region 1, which allow *C. cainii* to almost completely dominate. The adjacent farmland has had a clear influence on the condition of the river in Region 2. The influence that the extrinsic properties associated with this

condition has had on the ability of *C. cainii* to completely dominate over *C. tenuimanus* is an important factor that requires investigation. The extrinsic factors behind the reduced replacement of *C. tenuimanus* in Region 3, compared to Regions 1 and 2, are not yet fully clear. Investigation is required to determine what are the actual differences and should focus on aspects of the availability and quality of food and shelter and also the overall quality of the aquatic habitat, for example water condition. If the replacement mechanisms that have allowed *C. cainii* to replace *C. tenuimanus* are to be studied, as they occur in the wild, then the pools of the upper reaches are the only locations where this can be done.

3.4.2 Cane Break Pool Mark-Recapture

Key Findings

1. *C. cainii* has a possible greater growth rate than *C. tenuimanus*.
2. *C. cainii* has a possible earlier spawning and brood release than *C. tenuimanus*.
3. Differences in intrinsic factors between *C. tenuimanus* and *C. cainii* play an important role in the replacement mechanisms operating and can be influenced by extrinsic factors associated with Cane Break Pool.

Replacement Mechanisms in Cane Break Pool

Recording the change in size (RCL) of recaptured marron revealed that the *C. cainii* might have a greater growth rate (mm/day) compared to *C. tenuimanus*. A greater growth rate is an intrinsic factor that could contribute to the ability of *C. cainii* to replace *C. tenuimanus*. If female *C. cainii* are larger at the spawning period compared to the equivalent cohort of *C. tenuimanus*, due to a greater growth rate, they could be expected to have a greater potential of producing more offspring (Morrissy 1970, Beatty *et al.* 2003). This would begin from when two equivalent cohorts first reach maturity and would continue through successive spawning seasons, given that the greater growth rate of *C. cainii* is maintained.

The actual time of spawning and release of juveniles is another intrinsic factor that could exacerbate the decreased recruitment ability of *C. tenuimanus*. The sampling event conducted at Cane Break Pool in mid December 2002 (Trip 7, see Figure 3.5) revealed the possibility of an earlier spawning period and hence earlier release of *C. cainii* juveniles from their mothers compared with *C. tenuimanus*.

If the suggested later spawning period of *C. tenuimanus* does exist, then, by the time of brood release in this species, *C. cainii* will have an immediate size advantage. Juveniles of *C. tenuimanus* are likely to be out competed in obtaining shared resources if they are released shortly after *C. cainii*, which would hold a continuous advantage in size over the proceeding months (Rorer and Capelli 1978). In aggressive interactions between juvenile crayfish it is nearly always the larger of the two that will win, which has been shown to hold true for several different species of crayfish (Bovbjerg 1970, Pavey and Fielder 1996, Issa *et al.* 1999). Winning can increase an individual's impending success as they become less likely to retreat in future encounters; continued losing can deter an individual from further fights (Pavey and Fielder 1996, Issa *et al.* 1999, Gössmann *et al.* 2000). Smaller juveniles will start losing and continue to lose (tendency to retreat) while winners that are dominant for extended periods escalate to higher intensities early in aggressive encounters, which gives them a better chance of winning (Pavey and Fielder 1996, Issa *et al.* 1999, Gössmann *et al.* 2000). Within a species this could be expected to be a normal process of naturally larger juveniles establishing dominance and being better able to procure resources. However, in the case of *C. tenuimanus* they will be at the disadvantage of having to compete with the excess of larger *C. cainii* juveniles, which are most likely trying to obtain the same resources.

If female *C. cainii* do release their broods earlier, then size differences will likely be maintained and possibly amplified as the equivalent cohorts age, due to the apparent greater growth rate of *C. cainii*. The apparent earlier recruitment of *C. cainii* into the catchable size range, depicted in the size/frequency histograms of Figure 3.7, provides a possible example of this. For reasons explained above, this could lead to a continued greater offspring potential of *C. cainii* in comparison to the equivalent cohort of *C. tenuimanus*, which could be expected to relate to a continued greater recruitment potential. This could easily translate to a progressive increase of *C. cainii* within populations compared to *C. tenuimanus*, over successive generations.

Other comparisons between *C. cainii* and *C. tenuimanus* did not reveal any obvious differences in intrinsic factors. The average sizes (RCL) of recaptured marron for both species did not have any relationship with their estimated growth rates (Figure 3.5). The likely reason was the relatively large size of most recaptured marron. The growth rate of marron, like other crayfish, should decrease as they get older, due to increasing intervals between moults. Depending on the time interval, most recaptured marron that did increase in size are likely to have moulted only once or twice during that period, due to their relative initial large sizes (Morrissey 1970, 1976, 1990). Also,

the use of a continuous growth rate measure, like the mm/day used, could be considered an arbitrary value for crayfish due to their intermittent growth periods. However, as an overall mean measurement for a comparison between two groups, i.e. *C. cainii* and *C. tenuimanus*, it does serve a purpose.

Neither species showed any relationship between the recapture interval and the displacement between marking and recapture. Due to the length of recapture intervals, often quite large, it is likely that marron of both species could have travelled continually throughout Cane Break Pool during this period. Some marron were recaptured at the opposite end of Cane Break Pool to where they were originally captured and marked, while others appeared not to have moved at all. Movement of crayfish within populations can vary greatly with respect to size, sex, upstream or downstream, time of year and species (Robinson *et al.* 2000, Byron and Wilson 2001, Light 2003). In order to find potential differences in intrinsic factors that may be considered to influence replacement, it would be better to continuously track the movement of *C. cainii* and *C. tenuimanus* to give a measure of movement, rather than displacement.

The recreational marron fishing ban imposed by the Fisheries Department of Western Australia for the 2003 season on the Margaret River, upstream of the junction with Ten Mile Brook, may have already had an influence on the population structure at Cane Break Pool. Based on the results of Trip 3 (24/4/02) and Trip 7 (7/5/03) it appears that the ban on recreational fishing has increased the proportion of larger sized marron, in particular those above the legal size (76 mm RCL). This finding may not be overly conclusive due to the difference in sample size between Trip 3 and Trip 7 (Figure 3.5), and the inherent variations that can exist in using baited traps, but it does provide a possible insight into the effects of the ban on recreational fishing.

It is possible that the population reductions caused by recreational marron fishing has helped *C. cainii* replace *C. tenuimanus* by creating populations that are influenced greatly by year-to-year recruitment. In a study of the mechanisms behind the replacement of the North American crayfish *Orconectes sanborni* by the introduced *O. rusticus*, Butler and Stein (1985) concluded that the capacity of *O. rusticus* to recover more rapidly from potential population reductions was due to a higher production and growth rate of young, and this was a major contributing factor in this species' ability to replace *O. sanborni*. If *C. cainii* does have greater population growth ability (intrinsic) compared to *C. tenuimanus* then recreational fishing (extrinsic: predation) is likely to have contributed to the rapid replacement of this native species in Cane Break Pool and throughout the Margaret River.

An increase in the proportion of marron above the legal 76 mm RCL for *C. cainii*, *C. tenuimanus* and hybrids could affect the replacement mechanisms operating. If larger marron remain in populations they might increase the recruitment potential for both species, as bigger females inherently have the capacity to produce more offspring (Morrissey 1970, Beatty *et al.* 2003). Although, if growth rates are maintained, then it could be expected that *C. cainii* will have the largest individuals and hence a greater offspring production potential. However, large size marron may also represent an extrinsic factor, in that they can be substantial predators of juveniles and smaller marron within populations (Morrissey 1976). The increased presence of large marron may decrease the recruitment potential for both species, which could ultimately favour *C. cainii*. Keeping the proportion of large marron at a reduced level may have actually helped slow the rate of replacement of *C. tenuimanus*, at least in Region 3.

The largest marron caught during sampling events for the mark-recapture study at Cane Break Pool were almost always *C. cainii*. The only time this was not the case was for Trip 5, although only eight marron in total were caught, and Trip 7, after the ban on recreational marron fishing. It is uncertain at present how the suspected increase in larger sized *C. cainii* and *C. tenuimanus* may alter the replacement mechanisms operating, but it can be expected that it will have some effect. However, all this assumes that the short period of legal recreational fishing accounts for a more significant amount of large sized marron removed from the population than does illegal poaching.

Summary of Cane Break Pool mark-recapture study

Although the results obtained from the mark-recapture study may not be overly conclusive, the species-specific properties they imply can translate readily into differences in intrinsic factors that could contribute to the rapid replacement of *C. tenuimanus* by *C. cainii* in the Margaret River. Given that these differences in intrinsic factors have also been used to explain the replacement of native crayfish by introduced species in other parts of the world, the theories developed from the results of this study may be valid, warranting further research. The association with extrinsic factors related to availability of resources (habitat quality) and predation pressure from recreational fishing and their influence on replacement has also been identified in this study. Overall, an avenue for directing studies into the replacement mechanisms operating in, and the continued monitoring of, Cane Break Pool, and other parts of the Margaret River, has been achieved.

Chapter 4: Synthesis and Management Recommendations



Collection tub containing *C. cainii* captured from the Margaret River.

4.1 Summary of Study Findings

4.1.1 Field Identification

Of the marron that were genetically identified, all those identified as *C. tenuimanus* in the field, based on morphology, were correctly identified. Any mistake regarding *C. tenuimanus* was found to be conservative in that no genetically identified hybrids or *C. cainii* were thought to be *C. tenuimanus*. The mistake of field identifying a marron as a hybrid, when it was actually *C. tenuimanus*, was minimal and not thought to be a significant problem. The production of a field identification guide to instruct members of the general public to accurately identify *C. tenuimanus* was found to be feasible due to the distinctiveness of this species' morphology. This guide will serve to educate people in correctly distinguishing between marron species found within the Margaret River. Once the guide has been demonstrated to be effective, it will enable community participation in active removal programs of *C. cainii* and hybrids, and ensure that minimal numbers of *C. tenuimanus* are accidentally taken during the recreational marron fishing season.

In respect to being able to reduce the time and cost of electrophoretic identification, by utilising the four allozyme marker loci, the field identification of hybrid marron was found to be insufficiently accurate. If the process of hybridisation within sympatric populations and its possible role in the replacement of *C. tenuimanus* by *C. cainii* is to be properly evaluated, then the collection of tissue samples will be needed. Different DNA techniques can be used to provide greater insights into the hybridisation process, as allozyme marker data cannot distinguish between F_2 and backcross hybrids, nor can parentage be determined.

4.1.2 Distribution Map

The distribution of marron species within the Margaret River was mapped and most of the remaining populations of *C. tenuimanus* were located. Only a small population of *C. tenuimanus* was found downstream of the Margaret River townsite; this species is restricted to the forested upper reaches where it only occurs in sympatry with *C. cainii*. The Margaret River is essentially dominated by this introduced species. There is an abrupt boundary of reappearance for *C. tenuimanus* between the agricultural land use of the middle reaches and the onset of State Forest. The proportion of *C. tenuimanus* changes from approximately 0 % to almost 50 % between neighbouring pools in farmland (site 12) and forest (site 13) respectively. Extrinsic factors associated with different locations, such as habitat quality and predation pressure, appear to affect

the distribution of *C. tenuimanus*. Further research is needed to evaluate the extent to which these different extrinsic factors may influence the effect that various differences in intrinsic factors between *C. tenuimanus* and *C. cainii* has on species replacement.

The Margaret River was divided on a regional scale comprising the lower (Region 1), middle (Region 2) and upper reaches (Region 3), which was based upon adjacent land use and the results of the distribution study. It is likely that each region will require differing management strategies in the conservation of *C. tenuimanus*. Region 3 will be the most important for immediate short-term management as these upper reaches are the stronghold of *C. tenuimanus* and the only place where its interactions with *C. cainii* can be studied in the wild.

4.1.3 Mark-Recapture at Cane Break Pool

The mark-recapture study carried out at Cane Break Pool has provided some insights into the possible intrinsic factors influencing the replacement ability of *C. cainii*. This included a possible greater growth rate of *C. cainii* and earlier spawning period, which suggests that *C. cainii* could have a greater recruitment potential than *C. tenuimanus*. This superior ability of *C. cainii* in population growth and recovery from reductions in population size, such as that caused by recreational fishing, and in combination with extrinsic factors mentioned above, could easily contribute to a progressive replacement of *C. tenuimanus*.

The results of this study were not overly conclusive, but the differences in intrinsic factors that were implied have been identified in other parts of the world as being influential in crayfish species replacement. For this reason, these differences in intrinsic factors, and other potential properties, warrant further investigation and could be vital in preventing the extinction of *C. tenuimanus*.

4.2 Replacement Mechanisms Affecting *C. tenuimanus*

4.2.3 Associations Between Intrinsic and Extrinsic Factors

The extent to which resources that juveniles of native and introduced species may be competing for are actually limited (an extrinsic factor), necessitating the need to compete for them, will determine the degree in which a greater recruitment ability (intrinsic factor) may serve as a replacement mechanism, in terms of higher juvenile survival (Butler and Stein 1985). It is very important for juvenile crayfish to procure shelter because of the risks from adults and other aquatic predators (Garvey *et al.* 1994, Söderbäck 1994a, Figler *et al.* 1999). When shelters are limited, larger and more aggressive crayfish have been shown to be superior in holding onto this resource, between species (Usio *et al.* 2001) and within (Figler *et al.* 1999). These are prime examples of extrinsic factors interacting with differences in intrinsic factors between species to influence replacement.

In a place such as Cane Break Pool, it is likely that important resources like food and shelter are not severely limited. It could be for this reason that, at present, *C. tenuimanus* still makes up a substantial proportion of the marron population at Cane Break Pool and other similar sites in Region 3. This is in direct comparison to lesser quality pools within this region and throughout Region 2. It is possible that this is an example of how, without the problem of limited resources such as food and shelter, differences in intrinsic factors related to life history can be the major contributors to replacement. The amount of time since introduction could also be considered an extrinsic factor that influences replacement of *C. tenuimanus*. The rate of replacement is likely to increase as differences in intrinsic factors related to life history allow *C. cainii* to have progressively more offspring recruited into populations. Therefore, the current situation in Region 3 is not expected to persist and can be reflected in the almost complete lack of *C. tenuimanus* in similar quality habitats in the lower reaches (Region 1), where it is presumed that *C. cainii* has had a longer presence.

A probable greater fishing pressure in Region 1, due to the close proximity of local people, could explain the absence of *C. tenuimanus* from these apparent high quality habitats. The interaction between the extrinsic factor associated with recreational fishing (i.e. predation) and the extrinsic factor related to a suspected longer *C. cainii* inhabitancy (i.e. time) might have influenced the almost complete replacement of *C. tenuimanus* from the lower reaches. Increased fishing pressure could have accentuated the potential ability of *C. cainii* for greater population growth, which then increased the

rate of replacement and decreased the time needed for *C. tenuimanus* to become locally extinct.

Extrinsic factors associated with habitats could also serve to accentuate differences in intrinsic factors, such as growth rate and size of marron present. Parkyn *et al.* (2002) demonstrated that differences in extrinsic factors between streams in native forest and pastoral land use, influenced the growth and population dynamics of the New Zealand freshwater crayfish, *Paranephrops planifrons*. Crayfish density was lower in pasture streams, but crayfish there had faster growth rates due to greater moult frequency and larger moult increments, and females reached maturity a year earlier. This same situation could be reflected in the agricultural land use within the middle reaches of the Margaret River. At site 10 in particular, some very large *C. cainii* were captured at an apparent low density. It could be possible that extrinsic factors associated with the middle reaches increased the replacement ability of *C. cainii* through increasing this species' growth potential. The large size obtained by *C. cainii* here might indicate an increased competitive ability over *C. tenuimanus* in lower quality habitat with possible lower availability of resources.

Although, the role that hybridisation plays as a replacement mechanism has not been addressed in detail, there are some associations that can be discussed. Knowing the proportion of hybrids present within sympatric populations, and how this varies between locations, can help in understanding the hybridisation process (Perry *et al.* 2001a, 2001b). Regardless of the production of hybrids, which from a conservation viewpoint can be regarded just as detrimental as additional *C. cainii*, hybridisation can be considered more important as reproductive interference for *C. tenuimanus*. The reduced recruitment potential of *C. tenuimanus* caused by inappropriate mate selection will likely play a greater role in the replacement of this species. This role is likely to be accentuated as the proportion of *C. cainii* increases within populations in that the frequency of inappropriate mate selection with respect to *C. tenuimanus* could be expected to increase (see Söderbäck (1994b) for a similar situation concerning *P. leniusculus* and *A. astacus*). Therefore hybridisation, acting as reproductive interference, can be predicted to increase the rate of replacement as the proportion of *C. cainii* within populations increases due to other interacting intrinsic and extrinsic factors. In sympatric sites that have a relative high proportion of hybrids, it could be possible that the rate of replacement is higher in these locations compared to other sympatric sites. As explained above, there could be a higher frequency of interspecific matings, which results in hybrids, due to a lower proportion of *C. tenuimanus*. Sites

with this type of hybrid and *C. tenuimanus* proportions could be considered areas of special concern that need immediate attention (see sites 1, 15, 16 and 20 in Figure 3.3).

The treating of time as an extrinsic factor influencing replacement serves a very important aspect in considering the viability of remaining populations of *C. tenuimanus*. The interaction that time has with differences in intrinsic factors can be influenced by other extrinsic factors associated with location that can affect the rate of replacement and ultimately the time to extinction for *C. tenuimanus*.

4.2.2 Conceptual Flowchart of *C. tenuimanus* Replacement

The interacting intrinsic and extrinsic factors suggested from this project that seem to influence the replacement of *C. tenuimanus*, are summarised in a conceptual flowchart (Figure 4.1) that is based on Figure 1.2. This flowchart aims to highlight areas of research required to find conclusive answers for the overall interacting replacement mechanisms operating in the Margaret River.

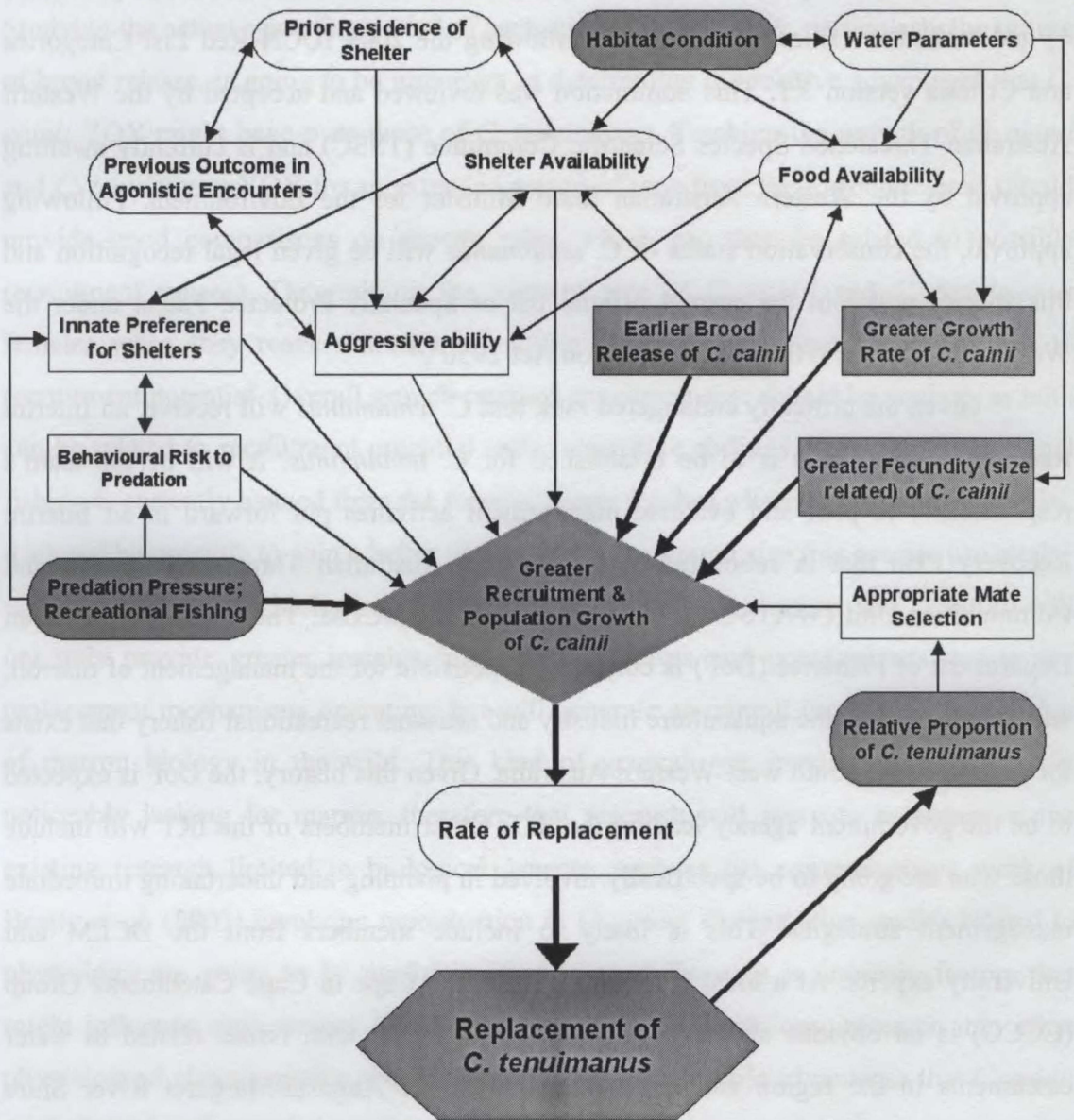


Figure 4.1. Conceptual flowchart of the intrinsic and extrinsic factors relating to the mechanisms operating in the replacement of *C. tenuimanus* by *C. cainii* (refer to Figure 1.2). The shaded components, and thicker arrows, represent the particular factors that have been identified and discussed in this project, in terms of their contribution in the replacement of *C. tenuimanus*. The remaining unshaded factors, and thin arrows, are those that still require investigation. This flowchart can be used to direct research into the affect that intrinsic (rectangles) and extrinsic (oblong circles) factors have on the replacement of *C. tenuimanus*, in relation to the likely greater recruitment and population growth ability of *C. cainii*. Immediate research should focus on the further investigation of the intrinsic and extrinsic factors identified in this project (shaded components) and how other connected factors might be influencing their effect. The role that particular intrinsic and extrinsic factors might be having on the replacement of *C. tenuimanus* is likely to vary within and between the different regions within the Margaret River. Following future research, this flowchart can be set up to represent replacement mechanisms operating in different regions, and locations.

4.3 Conservation of *C. tenuimanus*

4.3.1 Conservation Status of the Margaret River Hairy Marron

An official fauna nomination form for *C. tenuimanus* was submitted in January 2003 to the Western Australian Department of Conservation and Land Management (DCLM). Data obtained from the distribution study formed a significant component of this fauna nomination, which was for *C. tenuimanus* to be listed as threatened (Schedule 1) and ranked as critically endangered following the 2001 IUCN Red List Categories and Criteria version 3.1. This nomination was reviewed and accepted by the Western Australian Threatened Species Scientific Committee (TSSC) and is currently awaiting approval by the Western Australian State Minister for the Environment. Following approval, the conservation status of *C. tenuimanus* will be given legal recognition and this species will join the current official list of Specially Protected Fauna under the Western Australian Wildlife Conservation Act 1950¹.

Given the critically endangered rank that *C. tenuimanus* will receive, an Interim Recovery Team (IRT) is to be established for *C. tenuimanus*. It will be the team's responsibility to plan and evaluate management activities put forward in an Interim Recovery Plan that is submitted to the Western Australian Threatened Species and Communities Unit (WATSCU), which is part of the DCLM. The Western Australian Department of Fisheries (DoF) is currently responsible for the management of marron, which incorporates the aquaculture industry and seasonal recreational fishery that exists for marron in the south west Western Australia. Given this history, the DoF is expected to be the government agency leading the IRT. Other members of the IRT will include those who are going to be specifically involved in planning and undertaking immediate management strategies. This is likely to include members from the DCLM and University experts. At a local community level, the Cape to Cape Catchments Group (CCCG) is an obvious choice as member as they deal with issues related to water catchments in the region and are affiliated with the Augusta-Margaret River Shire Council.

¹ While this thesis was in draft form the nomination was formally accepted by the Western Australian State Minister for the Environment and *C. tenuimanus* was gazetted as critically endangered under the Western Australian Wildlife Conservation Act 1950 in July 2004.

4.3.2 Future Research Needed

The conceptual flowchart devised for the replacement of *C. tenuimanus* (Figure 4.1) can be used to direct future research into the interacting intrinsic and extrinsic factors affecting replacement of this species. Following on the possible differences in intrinsic factors related to growth rate and spawning that this project has suggested, a much more detailed study into the life history of both species should be undertaken. Studying the actual spawning period of each species in sympatry, particularly the timing of brood release, is going to be important in determining the relative advantages that *C. cainii* YOY might have over those of *C. tenuimanus*. Tracking the growth of *C. cainii* and *C. tenuimanus* YOY for an extended period of time from their brood release should provide good comparisons on growth rates, which can then be related to possible recruitment success. Determining the average size of *C. cainii* and *C. tenuimanus* females when they reach maturity is another intrinsic factor that can be related to recruitment potential. Overall growth rate and maximum size should be studied, as both can be related to recruitment potential and competitive abilities. Now that recreational fishing is currently banned from the forested upper reaches where *C. tenuimanus* occurs, it should be possible to gain a better picture of the maximum size this species can attain.

Research into biological differences between *C. tenuimanus* and *C. cainii* will not only provide greater insights into intrinsic factors and potential answers to the replacement mechanisms operating, but will generate an overall increase in knowledge of marron biology in the wild. This kind of general, yet important, knowledge is noticeably lacking for marron, therefore that research will serve to complement the existing research limited to biological aspects, such as the comprehensive work of Beatty *et al.* (2003) involving reproduction in *C. cainii*. Comparative studies related to physiology are going to be useful in identifying differences in intrinsic factors that might influence replacement. Apart from reproductive physiology, research into other physiological characteristics could provide answers to possible advantages that *C. cainii* might have in sub-optimal conditions and habitats.

Studies into differences in aggression between *C. tenuimanus* and *C. cainii* can provide clues to the competitive ability of each species for particular resources. This kind of study can be directly related to the influence that extrinsic factors could have on replacement, as the need to compete for limited resources will determine how important that competitive ability will be. Laboratory studies on aggressive interactions between freshwater crayfish should be compared directly with field observations to add strength to the results obtained (Bergman and Moore 2003).

Another interspecific interaction that should be studied is inappropriate mate selection and the role it might play in the replacement of *C. tenuimanus*. Determining the preference of males and females of both species for partners and how this affects the recruitment of *C. tenuimanus* will be valuable information. A greater insight into the hybridisation process and its influence on replacement can be achieved through such comparative studies. Genetic research can greatly help in the investigation of hybridisation through such studies as mitochondrial DNA analysis that can identify maternal heritage of F₁ hybrids (Perry *et al.* 2001a). The relative viability of hybrid marron and their competitive ability should also be considered an important aspect requiring investigation.

A much more in-depth study of the differences in extrinsic factors between locations in the Margaret River is required. Detailed analysis of factors associated with habitats such as shelter, including type and availability, water quality and food resources should be carried out. The extent to which resources are limited and the condition of habitats can then be quantified. This will greatly assist in removing the guesswork associated with trying to evaluate the extrinsic properties of particular locations. The influence of these properties on the replacement of *C. tenuimanus*, and the possible viability of this threatened species within its remaining habitats can then be better investigated.

The aspect of time is going to be a very important component of future research. Studies should essentially focus on determining which factors, both intrinsic and extrinsic, have the most influence on the rate of replacement of *C. tenuimanus*. As *C. tenuimanus* has suffered an extreme reduction in distribution and is under continuous threat from its close association with *C. cainii*, time is crucial in preventing the extinction of this unique endemic species. The outcomes of these studies will serve as a source in which to direct the overall management of the Margaret River for the basis of protecting *C. tenuimanus*.

Apart from the impact that the introduced *C. cainii* has had on *C. tenuimanus*, research will be needed on the possible impact this species has had on the entire Margaret River ecosystem. This includes native fauna such as freshwater fish and other *Cherax* species native to the Margaret River. It is unknown what effect *C. cainii* may have had on other native aquatic species through such potential impacts as habitat alteration, predation or even introduction of diseases. By conducting research into these potential impacts, it should be possible to develop an insight into the overall effect that *C. cainii* has had on the Margaret River ecosystem.

4.3.3 Short Term Management

Apart from the development of experimental studies into the replacement mechanisms operating, there are immediate actions that can and should be looked into for the conservation of *C. tenuimanus*. As time is a limited element that needs to be considered, these initial steps will serve to set up the recovery process and are likely to be inclusions in the development of the Interim Recovery Plan.

Public Awareness

First and foremost, the public needs to be made aware of the current conservation status of *C. tenuimanus*. The CCCG and Margaret River Regional Environment Centre can both serve as avenues for informing the local community about the plight of the hairy marron and how the introduced smooth marron threatens its existence. It is in this way that the production of an identification guide for marron in the Margaret River becomes particularly valuable. A guide, such as that in Figure 2.3, can be distributed by or through these local agencies to educate the public on the conservation status of *C. tenuimanus* and the identification of marron species. The DoF could also supply this, or equivalent, identification guide when issuing recreational fishing licences, informing those who might wish to travel to Margaret River from other locations during the marron season. With the distribution of a field identification guide, it should be feasible to reopen the middle and upper reaches to recreational marron fishing, which will hopefully benefit *C. tenuimanus* by decreasing the recruitment potential of *C. cainii*.

Active Removal of *C. cainii*

An obvious short-term management strategy in protecting *C. tenuimanus* would be the active removal of *C. cainii* from the Margaret River, especially where there are sympatric populations. This could be attempted in a number of ways and include the participation of the local community who hold a vested interest in the conservation of *C. tenuimanus*. Given that members of the general public are trained to identify correctly *C. tenuimanus* (i.e. the identification guide) it might be possible to hold a controlled 'fish-out' period. This could serve as another valuable method to raise public awareness, as recreational marron fishing is an already established past time and a 'fish-out' should draw plenty of attention. Having an open bag and size limited for *C. cainii* (and hybrids) and ensuring the persons participating know how to correctly identify *C. tenuimanus* and not to take this species potentially may be a valuable management strategy. However, restrictions will need to be applied on the number of persons taking part and

the capture methods used to ensure there is not an adverse effect on the local environment.

The timing of such an event should be considered when planning this kind of management strategy. Periods of peak winter flow should be avoided so as to reduce the risk of personal injury to participants. Planning this type of event for the time immediately prior to the marron spawning period may achieve the best outcome. Removing *C. cainii* before spawning is likely to severely impact on their recruitment, which can only be to the benefit of remaining *C. tenuimanus*. Attempting active removal of *C. cainii* during the actual spawning period may not be effective enough as females are generally inactive and difficult to capture (see (Morrissey 1970)). There is also the chance of interfering with female *C. tenuimanus* through their possible capture, which could cause the loss of eggs or newly hatched juveniles (see (Morrissey 1970)). The removal of *C. cainii*, of at least legal size (76 mm RCL), can re-commence during the recreational marron fishing season.

Removal of *C. cainii* from the sympatric populations of the upper reaches could pose a problem for ongoing studies being undertaken that are looking at the interspecific interactions. Risking the continued replacement of *C. tenuimanus* by *C. cainii* from populations for the sake of research may be hard to justify when the benefits of removing the major threatening process (*C. cainii*) are easily envisaged. However, it should be possible to incorporate removal of *C. cainii* into some of these studies by monitoring how this species might recover following removal. This will be very useful in looking at the rate *C. cainii* can replace *C. tenuimanus*. A continued monitoring protocol should be established before any active removal of *C. cainii* proceeds, including that to be carried out by members of the general public.

Reintroduction of *C. tenuimanus*

Active removal of *C. cainii* from the Margaret River on its own, particularly in regions where *C. tenuimanus* does not occur, may not serve as an effective management strategy. Incorporating the reintroduction of *C. tenuimanus* into areas it does not currently inhabit, following the removal *C. cainii*, is likely to produce better results. The source of these *C. tenuimanus* will undoubtedly need to come from captive populations. The DoF already has an established captive population of *C. tenuimanus* at their Southwest Freshwater Research Station in their Pemberton trout hatchery, Western Australia. This was originally set up in a single pond around 1985 when *C. cainii* (not yet recognised) was first recorded in the Margaret River, but was not intended for

reintroduction purposes (Horwitz 1994). Following some contamination of *C. cainii* in this population, either from the original wild stock or from other ponds at the hatchery, this captive population program underwent re-evaluation (Horwitz 1994). The DoF has, however, founded another captive population of *C. tenuimanus* at the hatchery from individuals captured in the wild during 2003 (Molony, pers. comm.). This was apparently following the recommendation made by Austin and Ryan (2002) that such a population should be established to act as a source of *C. tenuimanus* for reintroduction in the Margaret River.

In terms of short-term management, the availability of large numbers of *C. tenuimanus* for reintroduction may not yet be possible. Until a source, or sources, of *C. tenuimanus* to produce a substantial number of individuals for reintroduction has been established, the active removal *C. cainii* from sites lacking *C. tenuimanus* may need to be limited. There is the potential to disrupt local ecosystems due to the keystone role that freshwater crayfish can have within their aquatic habitats (Momot *et al.* 1978, Momot 1995, Nyström *et al.* 1996, Statzner *et al.* 2000, Parkyn *et al.* 2001). Although *C. cainii* is an introduced species it currently fills the ecological role that *C. tenuimanus* would have done in places where this native species is, at present, no longer found.

Regardless of the size of the captive population held by the DoF, other potential sources will need to be established, which is an appropriate short-term management action. A potential for establishment of captive populations is to use existing farm dams already within the Margaret River catchment. Local farmers should be encouraged to stock their dams with *C. tenuimanus*, with the captive population held by the DoF serving as the best source, so as not to impact on existing wild populations. Removing *C. tenuimanus* from the wild may be detrimental to the remaining population as it might cause an increase in the recruitment of *C. cainii* within these locations. Depending on the actual number of *C. tenuimanus* removed, there is the possibility that this newly vacated space is quickly occupied by an expanding *C. cainii* population. If *C. tenuimanus* is to be taken from the wild then this exercise should also involve the removal of a great deal more *C. cainii* at the same time. Once suitable *C. tenuimanus* numbers have been established within farm dams, they can then be used as a source for reintroduction of *C. tenuimanus* into the Margaret River.

Preventing Further Introduction of *C. cainii*

The use of farm dams brings about an important conservation issue in relation to actually preventing any continued introduction of *C. cainii* into the Margaret River. A

very important short-term management strategy is to identify which dams within the Margaret River catchment contain *C. cainii* and could potentially be acting as source of introduction. Once the location of these dams has been established, it will be necessary to persuade landowners to remove the *C. cainii* population. It is hoped they will be persuaded by being able to restock with *C. tenuimanus*. A potential method of persuasion could be the idea of community spirit in the knowledge that by removing *C. cainii* and replacing them with *C. tenuimanus* each landowner is contributing to the conservation of this unique species found only in the Margaret River. There is also the issue of the possibility that some farm dams may already contain populations of *C. tenuimanus*. If this is the case, then these populations may serve for immediate reintroduction programs, given the cooperation of landowners to exploit their hairy marron stocks.

One dam that may continue to be a potential source of *C. cainii* introduction into the Margaret River is Ten Mile Brook Dam (Figure 3.3, site 7). The complete removal of *C. cainii* from this large dam may be very difficult to achieve. There is also the chance that it may be continually stocked with *C. cainii* when water is pumped from the Margaret River to increase water in the dam. It is unlikely that Ten Mile Brook dam will be included in any community participation events, due to it being the town's water source. The risk of water contamination from people fishing for marron would prevent the Water Corporation (the dam's controlling body) from allowing such community conservation measures to take place. However, removal of large numbers *C. cainii* in a controlled manner and combining this with the introduction of *C. tenuimanus* into the dam could be a possible management strategy. The controlled removal of *C. cainii* may involve extensive trapping or drop netting under the guidance of members of the official recovery team for *C. tenuimanus*, particularly the DoF.

In line with the public awareness strategy is the need to educate people about not releasing any *C. cainii* into the Margaret River. This is something that may occur as people move from river to river during the recreational marron fishing season. Marron caught from other locations might be released into the Margaret River for such reasons as fear of being caught with undersized marron or in excess of the legal daily bag limit (10). It could be the intention of some people to 'restock' sections of the river to increase population sizes for future fishing trips. These introduced marron will undoubtedly be *C. cainii*, so the importance of ensuring such practice is stopped, in terms of preventing additional pressure on *C. tenuimanus*, can be clearly seen. There is also the issue of possible disease introduction from foreign marron, which is another

factor that needs to be avoided at all costs. This issue of disease risk will also need to be addressed in the use of farm dams for captive populations of *C. tenuimanus* for the purpose of reintroduction into the Margaret River.

River Restoration

Immediate restoration of degraded sections is a strategy that can set up the benefits of future reintroduction of *C. tenuimanus* and the long-term management of the Margaret River. It will be important to ensure that reintroduced *C. tenuimanus* can establish themselves and not be just as quickly replaced by *C. cainii*. Restoration of degraded areas may also allow natural recolonisation by *C. tenuimanus* following the removal of *C. cainii*. The CCCG (2003) have outlined recommendations to protect and improve the condition of the Margaret River in their action plan. They have focused on issues such as loss of native fringing vegetation and ongoing degradation of tributaries and the effect on water quality of the Margaret River. They also mention the obstruction to migration that the three weirs within the townsites pose to native fish. The native hairy marron receives a brief mention but is not a focus of this action plan. However, any kind of restoration that can improve the condition of the Margaret River in degraded sections is going to benefit the survival chances of *C. tenuimanus* in areas into which it may be reintroduced. Fish ladders have already been installed on one of the weirs for the purpose of aiding the upstream migration of native fish, including the pouched lamprey, *Geotria australis*. This was coordinated through the Margaret River Regional Environment Centre and is an example of community-led conservation that can also be utilised for the management of *C. tenuimanus*.

An example of a man-made structure, like the fish ladder, that could be used in the conservation of *C. tenuimanus* is the use of artificial shelters. It may take some time following river restoration programs of degraded sections for abundant shelter to become available. Shelter such as that created from large woody debris falling into the water will take time to accumulate both on land and in the water. It would be advisable to supplement the shelter availability, although they do not necessarily have to be artificial or man-made. Placing such things as large woody debris or rock accumulations from external sources could be an option.

If shelters are to be supplemented as part of a management strategy for *C. tenuimanus* then it should also be coordinated with removal and reintroduction programs. It would not be advisable to increase the shelter availability for a population containing only *C. cainii*, as this may allow them to increase in numbers making

removal more difficult. Competition for shelters is another factor to take into consideration in that there will need to be enough for both species in places where *C. tenuimanus* has been reintroduced following removal of *C. cainii*. It will be likely that not all the *C. cainii* in a particular location are successfully removed, and assuming they have a competitive edge over *C. tenuimanus* for shelters, it would be recommended to ensure that this resource is not limited.

The basis behind river restoration for the benefit of *C. tenuimanus* should centre on removing those extrinsic factors associated with an increased replacement ability of *C. cainii*. Ensuring that valuable resources are not limited will reduce the influence that this has on the replacement of *C. tenuimanus*, which is reflected in restricted occurrence of this species in the better quality habitats of the forested upper reaches. Without the restoration of degraded sections of the Margaret River, the success of *C. tenuimanus* reintroduction and *C. cainii* removal programs may be compromised. The extrinsic factors that have influenced the almost complete replacement *C. tenuimanus* from all but the forested upper reaches must be addressed and dealt with. River restoration is definitely one method of doing that effectively.

Continued Monitoring

Any short-term management strategies undertaken, such as those recommended above, will require continued monitoring so their success or failure can be assessed. Any progress not to the benefit of *C. tenuimanus* can be quickly identified and management plans can be adjusted or changed in an attempt to counteract such events.

Now that the distribution and relative proportion of the different marron species within the Margaret River has been mapped, there is the basis for continued monitoring of locations. Managers can document possible future changes in the distribution and proportion of marron species. This will provide the opportunity to determine the rate of replacement of *C. tenuimanus* by *C. cainii*, should this continue. More positively though is the opportunity to document the potential recovery of *C. tenuimanus* populations throughout the Margaret River, following the beginning of conservation for this threatened species. The ultimate success of short-term and long-term management strategies will be the establishment and recolonisation of *C. tenuimanus* in the middle and lower reaches of the Margaret River and a drastic reduction in the proportion of *C. cainii* within populations throughout.

4.4 Overall Conclusion

4.4.1 Recovery of *C. tenuimanus*

Now that *C. tenuimanus* is to be officially listed as threatened by the Western Australian State Government the opportunity for continued research and the development of recovery plans is present. The conceptual flowchart developed for the replacement of *C. tenuimanus* has identified areas of research still needed to fully understand the replacement mechanisms operating. What is clear is that this process is controlled by a mix of interactions between intrinsic and extrinsic factors, which together influence the rate and overall replacement of *C. tenuimanus* by *C. cainii*. Time has been identified as an influential extrinsic factor and can itself be considered a limited resource in the conservation of this threatened species. The recovery process will need to prioritise all the potential management strategies that will provide the most immediate and long-term advantage to *C. tenuimanus*.

Complete removal of *C. cainii* from the Margaret River may be impossible to achieve, which is why continued research into replacement mechanisms is important. An over-riding research imperative would be to focus on potential differences in reproductive biology and the factors aiding recruitment success for an understanding and management of the replacement mechanisms operating. Continued active removal of *C. cainii* and reintroduction of *C. tenuimanus* may be the best long-term option and will require immediate public awareness and community participation to be successful.

4.4.2 Community-Led Conservation

The threatened status of *C. tenuimanus* should be of great community concern as there is a risk of losing a unique form of marron found only in the Margaret River. Apart from the involvement of government departments and universities, there is an opportunity for the recovery process to be a community-led operation based on the potential 'flagship species' profile of *C. tenuimanus* (Nickoll and Horwitz 2000). The Cape to Cape Catchments Group (CCCG) will be the most suitable mediators of such a community-based recovery process and can provide directions and planning guidelines due to their local government association. Another locally based group that could serve to promote the conservation of *C. tenuimanus* is the Margaret River Regional Environment Centre, distributing information and promoting local activities.

The 'flagship species' profile provides the opportunity for an extensive conservation strategy involving the entire Margaret River catchment. In order to fulfil the goal of preserving *C. tenuimanus*, the restoration of degraded sections, to counter

the negative extrinsic factors associated with these locations that may affect the success of reintroductions, will be required. An overall restoration and protection strategy for the Margaret River will not only serve to help *C. tenuimanus* but also the many other native species that are found within this catchment. Conservation aimed at *C. tenuimanus* could benefit native fish and encompass protection of the whole catchment ecosystem, allowing the hairy marron to serve also as an 'umbrella species' (Roberge and Angelstam 2004). Focusing on providing the ultimate habitat possible for *C. tenuimanus* could involve the merging of different conservation programs such as livestock control, weed removal and water abstraction reduction. The management recommendations outlined in the CCCG's (2003) action plan for the Margaret River encompass restoration strategies that are aimed at protecting and improving the condition of this river. Incorporating the conservation of *C. tenuimanus* into existing recommendations should add strength to potential management plans developed by the CCCG. This idea equally applies in the development of management plans aimed directly at the conservation of *C. tenuimanus*.

From a regional perspective, the lower reaches (Region 1), due to the location of the Margaret River townsite (see Figure 3.3), will have focal importance for raising local community awareness and participation in the conservation of *C. tenuimanus*. Region 2 may require the greatest amount of effort in the conservation of *C. tenuimanus*, with river restoration the main focus due to the apparent association of this degraded habitat (CCCG 2003) with the increased replacement ability in *C. cainii*. Private land borders the river almost the entire length of this region so the cooperation of landowners will be essential. This cooperation will need to extend to all landowners within the Margaret River Catchment that have dams stocked with *C. cainii*, including Ten Mile Brook dam. Restocking these dams with *C. tenuimanus* following removal of *C. cainii* can provide for a greater community involvement through the availability of captive populations for reintroduction programs.

The forested upper reaches (Region 3) of the Margaret River is possibly the most critical region for the short-term conservation of *C. tenuimanus* species. This is the only region that has substantial populations of this threatened species, although only in sympatry with *C. cainii*. Public awareness of the current status of *C. tenuimanus* and the ability to correctly identify this species coupled with a carefully monitored to selectively remove *C. cainii*, will be the major actions in this region.

4.4.3 Contribution of this Project to the Conservation of *C. tenuimanus*

The current distribution map of marron species within the Margaret River has contributed greatly to the conservation of *C. tenuimanus*. This map formed an integral part of the official fauna nomination to the DCLM to have the hairy marron listed as a threatened species under the Western Australian Wildlife Conservation Act 1950 (Appendix 2). The almost completely restricted distribution of *C. tenuimanus* to the forested upper reaches, where it only occurs in sympatry with *C. cainii*, was the basis of ranking this species under the highest threatened category of 'critically endangered'. This listing has now brought about the formation of an Interim Recovery Team to be led by the Western Australian Department of Fisheries. The IRT will serve to develop an Interim Recovery Plan for *C. tenuimanus*, which will draw on the distribution map and other information from this project to begin immediate conservation actions. The map has provided a baseline for continued monitoring and the ability to evaluate the success or failure of management strategies that ultimately will be aimed at the recovery of *C. tenuimanus* in areas in which it currently does not occur and an increase in areas that it does.

The abrupt boundary of occurrence between agricultural landuse and the forested upper reaches has served to direct further research into the influence that extrinsic factors have on the rate of *C. tenuimanus* replacement. The importance of river restoration in the ability of *C. tenuimanus* to recolonise or become established following reintroduction, is clearly evident from the situation portrayed in the current distribution map of marron species within the Margaret River.

The results from the mark-recapture study at Cane Break Pool that suggested *C. cainii* might have a greater growth rate and earlier spawning period compared to *C. tenuimanus* require experimental confirmation. However, the increased recruitment potential that these findings have portrayed can be used to explain a progressive replacement of *C. tenuimanus*, even in locations where competition for resources is limited. In addition, these mechanisms have also been used to account for crayfish replacements in other parts of the world. Because of this, there is a strong basis for future research into the differences intrinsic factors between *C. tenuimanus* and *C. cainii*. This will not only provide insight into replacement mechanisms operating, but also increase the biological knowledge that is generally lacking for marron.

A very significant contribution to the recovery process has been the ability to produce a field guide for the accurate identification *C. tenuimanus* (Figure 2.3). This

field guide will serve several purposes in the conservation of *C. tenuimanus*; first and foremost, it will provide a means for educating people about the critically endangered status of this threatened species. The field guide will give members of the general public the ability to participate in community-led conservation, by training them in the identification of *C. tenuimanus*, which will be vital in active removal of *C. cainii*. Consideration may be given to the reinstatement of recreational fishing within the range of *C. tenuimanus*, provided that identification guides are issued with licences, so that people can distinguish between the two species and know not to take any hairy marron. Identification guides will also assist landowners in determining if they have farm dams stocked with hairy marron. The opportunity exists for these populations to be used for possible reintroduction of *C. tenuimanus* into the Margaret River, which is going to be a great asset in the conservation of this critically endangered species.

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APPENDIX 1: RECIPES USED IN THE STARCH GEL (SGE) AND CELLULOSE ACETATE GEL (CAGE) ELECTROPHORESIS

SGE

Gel recipe: 45 g Starch
360 ml deionised water
19.1 ml TEB buffer stock

TEB buffer stock: 109 g Tris
30.9 g Boric Acid
7.4 g Disodium EDTA
Made up to 1 litre with deionised water.

Electrode: Ratio of 1:5 for TEB buffer stock to deionised water.

Agar overlay: 4.0 g Agar
250 ml Deionised Water
Keep covered at 60 °C

Est stain recipe: 20 ml 0.1 M Tris Maleate Buffer
~2 ml α -Naphthyl Acetate Solution
~5 mg fast black salt
20 ml Agar Overlay

0.1 M Tris Maleate Buffer: 1.2 g Tris
1.2 g Maleic Acid
2.4 ml 1 M NaOH
Adjust pH to 5.3
Made up to 100 ml with deionised water.

α -Naphthyl Acetate Solution: 0.1 g α -Naphthyl Acetate
10 ml Acetone
10 ml Deionised Water

Peptidase stain recipe: ~5 mg peptide (leu-pro[#], leu-gly-gly[#] or leu-tyr*)
~5 mg O-Dianisidine diHCl
20 ml 0.1 M Sodium Phosphate Buffer
~5 mg Peroxidase
~5 mg L-Amino Acid Oxidase (add just before use)
20 ml Agar Overlay

0.1 M Sodium Phosphate Buffer: 30.5 ml 0.2 M Na_2HOP_4
19.5 ml 0.2 M NaH_2OP_4
Adjust pH to 7.0
Made up to 100 ml with deionised water.

*Dissolve leu-try in 2 drops of 0.1 M HCL along with O-dianisidine diHCl.

[#]Leu-pro and leu-gly-gly are added together in the same stain mixture.

Note: The amounts entered for ingredients in the stain recipes are mostly approximations. Once an amount has been initially measured it can then be visually approximated on the end of spatula.

APPENDIX 1: CONTINUED

CAGE

Tris Glycine (TG) pH = 8.5:

3.0 g Tris

14.4 g Glycine

Made up to 100 ml with deionised water.

This is a 10x concentration so dilute TG with deionised water for use in running tanks and soaking gels.

Est stain recipe:

2 ml 0.1 M Tris Maleate Buffer (see SGE procedure)

~200 μ l α -Naphthyl Acetate Solution (see SGE procedure)

~10 drops of saturated Fast Red TR salt solution

2 ml Agar Overlay (see SGE procedure)

Saturated Fast Red TR salt solution: Dissolve solid Fast Red TR salt in deionised water within a 1.5 ml ependorf tube until solution becomes saturated.

Peptidase stain recipe:

~0.5 mg peptide (leu-pro, leu-gly-gly or leu-tyr*)

~0.5 mg O-Dianisidine diHCl

2 ml 0.1 M Sodium Phosphate Buffer (see SGE procedure)

~0.5 mg Peroxidase

~0.5 mg L-Amino Acid Oxidase (add just before use)

2 ml Agar Overlay (see SGE procedure)

*Dissolve leu-try in 2 drops of 0.1 M HCL along with O-dianisidine diHCl.

Note: As with the SGE procedures the amounts entered for ingredients in the stain recipes are mostly approximations. For dry ingredients a tiny amount on the end of a small spatula can be visually approximated.

APPENDIX 2: FAUNA NOMINATION FORM SUBMITTED TO DCLM



DEPARTMENT OF CONSERVATION AND LAND MANAGEMENT

FAUNA NOMINATION FORM

Proposed addition, deletion or other change to listings of taxa Specially Protected pursuant to the Wildlife Conservation Act 1950 (threatened, presumed extinct or otherwise specially protected);

and/or,

amendments to the CALM Priority Fauna List.

See CALM Policy Statement No. 33 for criteria, definitions. Please complete all sections. Attach additional information, if space is insufficient.

1. TAXON (name) *Cherax tenuimanus* (Smith) 1912

2. CURRENT LIST/SCHEDULE: Threatened ☐ Presumed Extinct ☐
Other special protection ☐ Priority ☒ None ☐ (mark appropriate box)

3. PROPOSED LIST/SCHEDULE: Threatened ☒ Presumed Extinct ☐
Other special protection ☐ Priority ☐ None ☐

4. PROPOSED IUCN THREAT CATEGORY (see page 3): Extinct (EX) ☐ Extinct in the Wild (EW) ☐
Critically Endangered (CR) ☐ Endangered (EN) ☐ Vulnerable (VU) ☐ Lower Risk (LR) ☐

5. SUMMARY REASON FOR CHANGE:

More common than previously thought ☐ Name Change ☒
Taxonomic uncertainty ☐ Less common than previously thought ☒
Significant distribution change or habitat alteration ☒
Other ☐ (reason)

6. TAXONOMIC HISTORY:

Until very recently the freshwater crayfish, marron, was recognised as pertaining to a single species, *Cherax tenuimanus* that was first described by Smith (1912). Austin and Ryan (2002) detail the splitting of that taxon into two species: *C. tenuimanus* (Smith) 1912 and *C. cainii* Austin 2002. *C. tenuimanus* (hairy marron), the species being nominated, is endemic to the Margaret River in the south west of Western Australia and retains the original species name as Margaret River is the type locality and a hairy marron is the type specimen (held at the WA museum). *C. cainii* (smooth marron) is widespread throughout the south west of Western Australia and has been heavily translocated, including into the Margaret River. The translocation of the smooth marron (*C. cainii*) into the Margaret River and its proceeding interactions with the hairy marron (*C. tenuimanus*) has been important to the recent taxonomic change (Austin and Ryan 2002).

Austin, C. M. and Ryan, S. G. (2002). Allozyme evidence for a new species of freshwater crayfish of the genus *Cherax* Erichson (Decapoda: Parastacidae) from the south-west of Western Australia. *Invertebrate Systematics* 16: 357-367.

Smith, G. W. (1912). The freshwater crayfish of Western Australia. *Proceedings of the Zoological Society of London* 1912: 144-170.

7. RECENT SURVEY RESULTS:

All following information is from Bunn (in preparation)(see appendix):

There are no populations within the Margaret River that solely consist of *C. tenuimanus*, which is predominantly found in the upper reaches. There is a distinctive boundary of occurrence for *C. tenuimanus* between agricultural land and state forest in the upper reaches (see sites 10 and 11 in appendix). There is a lack *C. tenuimanus* individuals up to the onset of state forest, from here it only occurs in sympatry with *C. cainii*. *C. tenuimanus* exists at about an estimated 40% of the marron population over its range in the forests of the upper reaches, but proportions vary in the pools that it inhabits. The remaining marron consist of an estimated 50% *C. cainii* and 10% hybrids. The marron population in the lower reaches is comprised almost exclusively of *C. cainii*. Recent trap data indicates the presence of *C. tenuimanus* at about 1% of the marron population at sites downstream of the Margaret River town site. The *C. tenuimanus* individuals caught are either vagrants from their stronghold in the forests of the upper reaches or the offspring of parents that have persisted in these lower most reaches.

Bunn, J. J. S. (in preparation). The conservation status of the Margaret River hairy marron, *Cherax tenuimanus* (Smith) 1912 (Decapoda: Parastacidae). Masters Thesis, School of Natural Sciences, Edith Cowan University.

APPENDIX 2: CONTINUED

8. RESEARCH KNOWLEDGE/NEEDS:

What we know:

C. cainii was translocated into the Margaret River in the early 1980^a and has rapidly replaced the endemic hairy marron, *C. tenuimanus* throughout most of its original range (Austin and Ryan, 2002). *C. tenuimanus* is under threat of extinction through replacement by the translocated *C. cainii* and management measures are urgently required to prevent the loss of this species and the most significant genetic difference found within marron (Austin and Ryan, 2002, Nguyen *et al.*, 2002). Interbreeding is known to occur at low numbers and hybrids can be identified genetically from marker allozyme loci (Austin and Ryan, 2002, Bunn, in preparation). Preliminary studies show that *C. tenuimanus* can be readily identified from *C. cainii* and hybrids in the wild based on morphology (Bunn, in preparation). Studies are underway linking diagnostic genotypes to morphology for the purpose of producing a field identification guide to accurately distinguish between *C. tenuimanus*, *C. cainii* and possibly both F₁ and F₂ or backcross hybrids (Bunn, in preparation). This guide is intended for use by field workers needing to determine the proportions of marron types present at locations in the wild and eliminate the need to take tissue samples for electrophoretic analysis, hence saving time and money. The guide is also intended for members of the public to ensure they can correctly identify *C. tenuimanus*. This is so they do not remove *C. tenuimanus* when fishing or when involved in active removal of *C. cainii* and hybrids that may arise and include the local community. Mapping of the distribution of *C. tenuimanus* and *C. cainii* in the Margaret River is being undertaken to locate remaining populations of the hairy marron and highlight areas of conservation concern (Bunn, in preparation). A distinctive boundary appears to exist between agricultural land and forest for the occurrence of *C. tenuimanus* in the upper reaches.

What we need to know:

There is lack of knowledge of the basic biology of *C. tenuimanus* and how it compares to *C. cainii*. We need to know what behavioural, demographic and ecological factors have allowed *C. cainii* to rapidly replace *C. tenuimanus*. These displacement mechanisms need to be evaluated to determine the viability of *C. tenuimanus* within the Margaret River and provide insights into preventing the extinction of this species. Other threatening processes such as habitat loss/availability and water quality, which may be contributing to the success of *C. cainii*, will need to be identified. This will be important for potential reintroduction programs that may arise for *C. tenuimanus*. Explaining the distinctive boundary in occurrence of *C. tenuimanus* between agricultural land and forest in the upper reaches will highlight direct management actions needed for this species' conservation.

The potential of dams within the Margaret River catchment that contain *C. cainii* for the continued introduction of this species into the dominant tributary will need to be determined.

Nguyen, T. T. T., Meewen, S. Ryan, S. and Austin, C. M. (2002). Genetic diversity and translocation in the marron, *Cherax tenuimanus* (Smith): implications for management and conservation. *Fisheries Management and Ecology* 9: 163-173.

9. MANAGEMENT NEEDS & IMPLICATIONS:

Management will need to focus on the factors allowing *C. cainii* to rapidly replace *C. tenuimanus*. Before guidelines can be established the research outlined in the previous section needs to be undertaken to determine the displacement mechanisms and where to target management strategies.

Fishing for marron in the Margaret River will need to be managed specifically for the conservation of *C. tenuimanus*. Recreational fishing need not be banned all together but the local community and other fishers will need to be made aware of the two species of marron present and know not to take any of the endemic *C. tenuimanus*. Correct identification of *C. tenuimanus* will be of critical importance and is where the production of an identification guide (described in the previous section) will be most significant. Since the removal of threatening processes is integral in conservation strategies a relaxation of established marron fishing guidelines for *C. cainii* could be a management option. Community involvement in active removal of *C. cainii* will increase the public awareness for the conservation concern that exists for *C. tenuimanus*. With an increase in community knowledge *C. tenuimanus* will become an important flagship or icon species for restoration of degraded sections of the Margaret River. Marron have already been recommended as a flagship or icon species for restoration of the upper reaches of the Blackwood River (Nickoll and Horwitz, 2001).

Prevention of further introductions of *C. cainii* will be a crucial management need. Dams and marron farms that feed off the Margaret River and its tributaries will need to be regulated to ensure that no *C. cainii* that they contain can escape into the natural waterways. Destocking of farm dams containing *C. cainii* and then restocking with *C. tenuimanus* may be an option. From the planning stage any stock removal, or any direct restocking or reintroduction of *C. tenuimanus* must be carefully evaluated for potential impacts.

Nickoll, R. and P. Horwitz (2000). Evaluating flagship species for ecosystem restoration: a case study involving freshwater crayfish and a river in southwestern Australia. In J. L. Craig, Mitchell, N. and Saunders, D. A. (eds) *Nature Conservation 5: Nature Conservation in Production Environments: Managing the Matrix*, Surrey Beatty & Sons: 557 - 564.

APPENDIX 2: CONTINUED

10. DISTRIBUTION BY CALM REGION:

Kimberley ☐ Pilbara ☐ Midwest ☐ Goldfields ☐ Wheatbelt ☐ Swan ☐
 Central Forest ☐ Southern Forest ☒ South Coast ☐

11. KNOWN POPULATIONS AND RANGE:

A. Conservation Reserves (National Parks, Nature Reserves, Marine Parks, State Forests)

Forest Estates, two sections

The Rapids Conservation Park

Cane Break Pool CALM camp site (site 15 in appendix)

State Forest

Reserves alongside pools downstream of the town site that contain *C. tenuimanus* at low proportions (sites 1-3 in appendix).

B. Other Crown Lands

Some vacant crown land adjacent to pools downstream of the town site that contain *C. tenuimanus* at low proportions (sites 1-3 in appendix).

C. Private/Leasehold Lands

Some alongside pools downstream of the town site that contain *C. tenuimanus* at low proportions (sites 1-3 in appendix).

The range of *C. tenuimanus* is mostly limited to the forests of the upper reaches (sites 11-19 in appendix) with the species also occurring at very low proportions in pools downstream of the Margaret river town site (sites 1-3 in appendix) (Bunn, in preparation).

12. TRENDS IN POPULATION SIZE & RANGE

A. Previous

Prior to the introduction of *C. cainii* in the early 1980's *C. tenuimanus* was found throughout the Margaret River and was the only species of marron present (Austin and Ryan, 2002).

B. Current

The distribution of *C. tenuimanus* has been greatly reduced and is now predominantly found in the forests of the upper reaches (sites 11-19 in appendix, with site 19 being the extent of the permanent pools) indicating a reduction in population range of around 70-80% (Bunn, in preparation) (see appendix). Exact population size is difficult to estimate due to the differences in catchability of individuals based on size, sex and time of year (Morrissey, 1970, 1974), but it occurs at about 40% of the captured marron over its remaining range (see section 7. above). Going by the aforementioned information it can realistically be estimated that there has been an approximate 80-90% reduction in the population size of *C. tenuimanus* since the introduction of *C. cainii* into the Margaret River.

Morrissey, N. M. (1970). Spawning of marron, *Cherax tenuimanus* Smith (Decapoda: Parastacidae) in Western Australia. *Fisheries Bulletin, Department of Fisheries and Fauna Western Australia* 10: 1-23.

Morrissey, N. M. (1975). The influence of sampling intensity on the 'catchability' of marron, *Cherax tenuimanus* (Smith) (Decapoda: Parastacidae). *Australian Journal of marine and Freshwater Research* 26: 46-73.

APPENDIX 2: CONTINUED

13. SUMMARY STATUS ASSESSMENT:

Summary from the IUCN red list categories and criteria version 3.1.

A 2: Population size reduced by >80% (**CR category**) and causes have not ceased and are not yet fully understood based on **a** (direct observation), **c** (a decline in the area of occupancy, extent of occurrence and/or quality of habitat) and **e** (the effects of introduced taxa, hybridisation, pathogens, pollutants, competitors or parasites).

Evidence: See section 12B. above.

B 2: Area of occupancy <10 km² (**CR category**). The pools that *C. tenuimanus* inhabits in the forests of the upper reaches (sites 11-19 in appendix) and the pools downstream of the town site (sites 1-3 in appendix) would equate to much less than 10 km².

a: Exists at only one (**CR category**) location, the Margaret River.

b: Continuing decline in (i) extent of occurrence, (ii) area of occupancy, (iv) number of locations or subpopulations and (v) number of mature individuals. If the processes that have enabled *C. cainii* to replace *C. tenuimanus* from most of its original range are allowed to continue then the aforementioned categories can all be expected to decline. This is particularly applicable to pools that *C. tenuimanus* inhabits downstream of the town site at such low proportions (Bunn, in preparation). Once these small populations are lost the pools in the forests of the upper reaches will be the only places that this species is found in the wild.

C 1: Population estimated to number <10 000 (**VU category**) mature individuals and with at least a 25% (**CR category**) estimated decline within three years.

See E below.

D 2: Population has a very restricted area of occupancy such that it is prone to the effects of human activities or stochastic events within a very short period of time in an uncertain future, and is thus capable of becoming Extinct in a very short period of time.

As the remaining populations of *C. tenuimanus* are restricted to the pools in the forests of the upper reaches then any adverse events such as chemical contamination or severe summer drought could be catastrophic for these localised populations.

E: Probability of extinction in the wild is at least 50% (**CR category**) within ten years.

This is based on the fact that the proportion of *C. cainii* increased from approximately 10% to 80% at a single location within the Margaret River from 1985 to 1992 and that sampling of the same site in 1998 revealed no *C. tenuimanus* to be present (Austin and Ryan, 2002). Given that *C. tenuimanus* now only occurs in sympatry with *C. cainii*, which is at approximately 50% of the marron population (Bunn, in preparation) (see appendix) it can be reasonably predicted that the extinction of *C. tenuimanus* in the wild could occur within ten years.

The Margaret River hairy marron, *C. tenuimanus* is critically endangered and under severe threat of extinction through replacement by the translocated marron, *C. cainii*.

14. PROPOSED BY:

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DATE: / /

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Note: The appendix referred to in this form is the same distribution map featured in Figure 3.3. The exception being that sites 7 and 12 had not been sampled at the time of producing this fauna nomination form. The site numbers referred to in this nomination form will need to be adjusted accordingly to match Figure 3.3.

APPENDIX 2: CONTINUED

Ranking (CALM Policy Statement No. 50) according to IUCN (2000) Red List Categories and Criteria (see IUCN (2000) for full details). Circle the relevant criteria and add notes to new page with any extra details as required.

IUCN RED LIST CATEGORIES AND CRITERIA VERSION 3.1

	CRITICALLY ENDANGERED	ENDANGERED	VULNERABLE
A) REDUCTION IN POPULATION SIZE BASED ON ANY OF 1) An observed, estimated, inferred or suspected population reduction of _____, over the last 10 years or 3 generations, whichever is the longer, where the causes are clearly reversible AND understood AND ceased, based on a, b, c, d or e 2) An observed, estimated, inferred or suspected population reduction of at least _____ over the last 10 years or 3 generations, whichever is the longer, where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible based on a, b, c, d or e 3) A population size reduction of _____, projected or suspected to be met within the next 10 years or 3 generations, whichever is the longer (up to a maximum of 100 years) based on a, b, c, d or e 4) An observed, estimated, inferred or suspected population reduction of _____ over any 10 year or 3 generation period, whichever is the longer (up to a maximum of 100 years in the future) where the time period must include both the past and the future, and where the reduction or its causes may not have ceased OR be understood OR may not be reversible, based on a, b, c, d or e a) direct observation, b) an index of abundance appropriate for the taxon, c) a decline in area of occupancy, extent of occurrence and/or quality of habitat, d) actual or potential levels of exploitation, e) the effects of introduced taxa, hybridisation, pathogens, pollutants, competitors or parasites.	≥90% ≥80% ≥80% ≥80%	≥70% ≥50% ≥50% ≥50%	≥50% ≥30% ≥30% ≥30%
B) GEOGRAPHIC RANGE IN THE FORM OF EITHER B1 OR B2 1) Extent of occurrence _____ and estimates indicating at least 2 of a-c 2) Area of occupancy _____ and estimates indicating at least 2 of a-c (a) Severely fragmented or known to exist at no more than _____ locations (b) Continuing decline, observed, inferred or projected, in ANY of the following: (i) extent of occurrence, (ii) area of occupancy, (iii) area, extent and/or quality of habitat, (iv) number of locations or subpopulations, (v) number of mature individuals. (c) Extreme fluctuations in any of the following: (i) extent of occurrence, (ii) area of occupancy, (iii) area, extent and/or quality of habitat, (iv) number of locations or subpopulations, (v) number of mature individuals.	<100 km ² <10 km ² one	<5 000 km ² 500 km ² five	<20 000 km ² <2 000 km ² ten
C) POPULATION ESTIMATED TO NUMBER _____ MATURE INDIVIDUALS AND EITHER 1) An estimated continuing decline of at least _____ within three years or one generation whichever is the longer (up to a maximum of 100 years in the future) OR 2) A continuing decline, observed, projected, or inferred, in numbers of mature individuals AND at least one of a-b a) population structure in the form of one of (i) no subpopulation estimated to contain more than _____ mature individuals) OR (ii) at least 90% of mature individuals in one subpopulation	<250 25% 50	<2 500 20% 250	<10 000 10% 1 000
D) (CR AND EN) POPULATION SIZE ESTIMATED TO BE LESS THAN _____ MATURE INDIVIDUALS D) (VU ONLY) POPULATION VERY SMALL OR RESTRICTED IN THE FORM OF EITHER 1) population estimated to number less than _____ mature individuals. OR 2) population with a very restricted area of occupancy (typically less than 20 km ²) OR number of locations (typically five or fewer) such that it is prone to the effects of human activities or stochastic events within a very short period of time in an uncertain future, and is thus capable of becoming Critically Endangered or even Extinct in a very short time period.	50 not applicable not applicable	250 not applicable not applicable	not applicable 1000 applies
E) QUANTITATIVE ANALYSIS SHOWING PROBABILITY OF EXTINCTION IN THE WILD IS AT LEAST _____	60% within ten years or three generations, whichever is the longer (up to a maximum of 100 years)	20% within 20 years or five generations, whichever is the longer (up to a maximum of 100 years)	10% within 100 years

PLEASE FORWARD COMPLETED FORM TO:

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